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BULLETIN OF
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MAY 1948

THE USE OF RADIOACTIVE IODINE IN THE DIAGNOSIS AND TREATMENT OF THYROID DISEASES*

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RADIOACTIVE iodine made its appearance on the scene in 1935 shortly after the discovery of artificially radioactive isotopes by Joliot and Curie[1] in 1934. Physics thereby provided biology and medicine with a new approach to their problems—a new instrument with which to attack them.

Because the thyroid has a specific avidity for iodine, labelled or tracer, iodine obviously can be employed as a means of investigating many aspects of thyroid physiology, normal and morbid, and consequently as an aid also, in the diagnosis of certain disturbances of thyroid function. Furthermore, because we can implant, by means of radioactive iodine, a source of radiation directly within the thyroid parenchyma, one can utilize this agent as irradiation therapy in certain thyroid diseases.

It is not within the scope of the present paper to discuss the use

* From the Thyroid Clinic of the Massachusetts General Hospital.
Given 4 December 1947 at the Stated Meeting of The New York Academy of Medicine.

of radioactive iodine in the field of research, although it may be mentioned in passing that the writer at the time of its introduction, predicted that its usefulness in this field would be greater than in that of therapeutics. Now after an elapse of ten years it can be said that the usefulness of radioactive iodine in thyroid research has been firmly established, but that its position in therapeutics is still in need of more evaluation.†

Diagnostic Uses

Let us first consider the role of radio iodine in diagnosis. There are three maneuvers that can be employed:

1. *Measurement of the excretion of labelled iodine* following the administration of a standardized tracer dose.
2. *Detection of the location* of administered radio iodine in the body, and its concentration by means of externally applied counters.
3. *The autoradiogram* which is the photo print of a section of tissue containing radioactive material brought in direct contact with photo sensitive film.

The determination of the excretion of radio iodine after the giving of a standard tracer dose is chiefly of use as a diagnostic procedure and as a check on therapy, in hyper- and hypothyroidism. It is a test indirectly of the avidity of the thyroid for iodine, and therefore, except when an anti-thyroid agent is operating, of the degree of stimulation of the thyroid and the kidney, and to a much less extent other tissues are in competition for it. One may assume with some degree of assurance that the thyroid takes up what iodine it requires and leaves the rest for excretion by the kidney. Both Keating, Power, Berkson and Haines[3] at the Mayo Clinic, and Skanse[4] at the Massachusetts General Hospital, have determined the curves of excretion of radio iodine following tracer doses at six hour periods up to 48 hours, at which time plateaus are usually reached. In thyrotoxic persons the excretion curves are lower and reach their plateaus sooner than in the euthyroid. The total urinary excretion of radio iodine in a 48-hour period is much less in thyrotoxic subjects than in euthyroids. In myxedema total excretion is more than in euthyroids, though the rate of excretion may be slower.

† A complete review of the use of radio iodine as a tool in the study of thyroid physiology will be found in the paper of Rawson and McArthur,[2] 1947.

If now we make the assumption that iodine not excreted in the urine is for the most part trapped by the thyroid, we may regard excretion tests as equivalent to tests of iodine collection by the thyroid. Keating and his co-workers[3] have made a careful analytical study of the renal component in excretion, and have shown that impaired renal function will cause retention of iodine quite as much as trapping by the thyroid. However, from the practical diagnostic point of view, in the absence of obvious renal impairment and recent iodine medication the excretion of radio iodine can be interpreted as a dependable index of one function of the thyroid, that of collecting iodine.

Hertz and Roberts[5] in 1942 observed the urinary excretion of tracer iodine over a 72-hour period in six classic cases of Graves' disease and in one normal subject. In the former excretion varied between 10 per cent and 37 per cent of the tracer dose, and in the latter it was 62 per cent.

At the present time the technique employed at the Massachusetts General Hospital, and devised by Dr. Skanse, is as follows:

A tracer dose of 100 microcuries of I^{131}(eight day half life) with 100 micrograms of inert sodium iodide as a carrier, is made up to 100 mls. One ml. is saved as a standard. The dose of 99 mls. is given orally, in the morning before breakfast, and all the urine is collected in two 24-hour periods. For the determination of the radioactivity, both of the material administered and of the urine excreted, the physician is dependent on the physicist, unless he can take the time (and it will be a long time) to become sufficient of a physicist to make them for himself. The results of the test obtained at the Massachusetts General Hospital by Skanse in three groups of cases is as follows:

1. The 48 hour excretion for 25 thyrotoxic patients varied between 6 per cent and 32 per cent, with an average of 19 per cent.
2. That for 15 euthyroid patients (controls) varied between 52 per cent and 84 per cent, with an average of 66 per cent.
3. That for six myxedematous subjects varied between 72 per cent and 91 per cent.

Such results are of course what could be expected. The hyperplastic thyroid, unless it be a frustrated hyperplasia, as when an antithyroid agent, thiouracil, for example, is being received, is making hormone at a rate increased over normal, and consequently must have an increased supply of iodine. In clinical hypothyroidism the converse

holds, and we find reduced collection, or indeed in full blown myxedema, no collection of iodine by the thyroid at all. Of course in full blown myxedema the thyroid may be completely atrophic.

We are asked not infrequently, which is the best index of thyroid function—basal metabolic rate, protein bound iodine level of the blood, or radio iodine excretion. Inasmuch as determination of these several values test different aspects of thyroid function, it is idle to attempt to say that any one of them is better than another. If one could always have all three, one would have a more complete picture of the morbid physiology of the patient than with but one or two. But practically, this is often impossible. Basal metabolic rate can be looked on as an index of the impact of thyroid hormone on all its end organs in the body. Its magnitude will depend both on the amount of hormone delivered and on the sensitivity of end organs to it. Protein bound iodine of the blood is an index of the level of circulating thyroid hormone and radio iodine excretion, indicating, as it does, iodine collection, is an expression of the thyroid's hunger for iodine.

In interpreting the significance of these tests in clinical cases, the points first mentioned must be kept in mind. If for convenience we speak now of radio iodine collection instead of excretion, we can say that, usually, the three values, basal metabolic rate, protein bound iodine of the blood, and radio iodine collection are all elevated in hyperthyroidism and all depressed in hypothyroidism. There are, however, some exceptions, and they are of diagnostic significance.

For example, when a hypo- or euthyroid person takes thyroid enough to produce hyperthyroidism, the basal metabolic rate and protein bound iodine are elevated, as in spontaneous thyrotoxicosis, but iodine collection is normal or depressed. Such a formula is pathognomonic of what we may call thyrotoxicosis factitia. We have detected some cases of it in persons who, being somewhat psychopathic, were taking thyroid surreptitiously. The explanation of the findings is not difficult. The basal metabolic rate is elevated because the body doesn't distinguish between an excess of thyroid hormone derived from the subject's thyroid gland, and one received per os. Also administered thyroid is reflected in the blood by elevation of protein bound iodine, as would be also autogenous hormone. But when it comes to iodine collection there is a difference between the effects of autogenous and administered hormone. When the body is flooded with thyroid hor-

mone from the subject's own thyroid, his thyroid obviously is hyperfunctioning and his iodine collection is increased. When an individual receives thyroid hormone from without, his thyroid is under no extra stimulation—indeed it may actually be inhibited, therefore his iodine collection is not raised, it may even be reduced.

Another interesting example of the diagnostic value of combined tests of thyroid function is the case of a woman with persistently elevated basal metabolism and some symptoms suggestive of thyrotoxicosis. Her radio iodine collection and protein bound iodine of the blood were quite normal. She came to autopsy finally, and proved to have a phaeochromocytoma, a hyperfunctioning tumor of the adrenal medulla. She had an excess of a calorigenic hormone which raised her basal metabolic rate, but it was adrenalin, not thyroxine, so iodine collection and hormone iodine in the blood were not elevated.

Detection of ingested radio iodine by means of Geiger-Müller counters applied to the surface of the body has been used by Hamilton and Soley as an in vivo technique for rough quantitation of per centage uptake by the thyroid in patients with various types of goiter. Collection curves were obtained, comparable to those got by excretion studies. Normal persons' excretion rose quite rapidly and reached plateaus in about 48 hours. The curves of thyrotoxic patients rose still more rapidly to reach peaks in about six hours. The radioactivity over the thyroid was much greater at that point than in normal subjects, but the activity then returned to about the same levels as those reached by normals.

External localization of radioactivity is also proving of great use in diagnosis in the field of thyroid malignancy. In general, thyroid tumors take up iodine in inverse ratio to their degree of malignancy. The determination of radioactivity over the surface of a goiter may give some clue to its probable nature. Also when an attempt at total thyroidectomy for malignancy has been made, this technique may be employed to determine whether any thyroid tissue remains in the neck. Metastases, if they have any iodine collecting power, and are sufficiently near the surface of the body, may be located by the counter.

The autoradiographic technique with radio iodine for studying the thyroid was introduced in 1940 by Hamilton, Soley and Eichorn.[6] These investigators allowed sections of thyroid tissue removed surgically from patients who had received tracer doses of radio iodine to

photograph themselves by bringing them into direct contact with photo-sensitive film. By this method they were able to show that hyperplastic tissue has a greater ability to concentrate iodine than tissue from euthyroid subjects, and that cancerous thyroid tissue had little or no such ability. Leblond[7] has modified the autoradiographic technique by making a solution of the photo-sensitive emulsion and painting it directly onto his microscopic section of tissue. In this way his areas blackened by irradiation remain in permanent juxtaposition to histologic detail. However, the histology is obscured somewhat by this process. The older method is to lay the autoradiogram over the stained sections and view them together macroscopically or microscopically.

In the field of diagnosis the autoradiogram with iodine has been exploited extensively in our clinic by Dobyns and Lennon.[8] For over a year now the aim has been to give tracer doses of iodine to all patients coming to operation for nodular goiter. The autoradiograms of thyroid tissue so obtained add to the precision of diagnosis of thyroid function, which may be quite different in one part of the gland from what it is in another. As previously shown by Cope, Rawson and McArthur[9] by chemical methods, there exist situations all the way from that of goiters in which a single adenoma possesses all the iodine collecting function, and the remainder of the gland none, to that of goiters in which adenomas are functionless and the remainder of the gland does all the iodine collecting. The use of the autoradiogram has facilitated, and refined this diagnostic approach. To date Dobyns and Lennon have made a total of 93 such studies. Particularly interesting is the use of the procedure to differentiate in puzzling cases between hyperfunctioning adenomas and true Graves' disease. It is also of value in the study of functioning cancers, both parent tumors in the thyroid and metastases elsewhere, if and when it is possible and proper to perform biopsies on them.

Therapeutic Uses

The application of irradiation to the thyroid for therapeutic purposes is far from new. Röentgen rays were so used as early as 1900, when Beck reported some observations on the Röentgen treatment of nontoxic goiter. From then on until the introduction of iodine by Plummer in 1923, the use of Röentgen rays in the treatment of thyrotoxicosis came into steadily increasing vogue. Studies on it were reported from

our own clinic in 1917 by Means and Aub[10] and in 1922 by Means and Holmes.[11] Radium also, or radium emanation, has been implanted in the thyroid, but never became a commonly used procedure. Röentgen irradiation, of course, has long been used for cancer of the thyroid, and in certain cases still is indicated, and finally, radio iodine has been used in the treatment of thyroid diseases, both toxic goiter and cancer.

In the paper from the Thyroid Clinic of the Massachusetts General Hospital by Hertz, Roberts and Evans[12] in 1938, the suggestion was made that it was "logical to suppose that when strongly active materials are available the concentrating power of the hyperplastic and neoplastic thyroid for radioactive iodine may be of clinical or therapeutic significance."

The therapeutic effect of all forms of radio therapy of the thyroid is due to destructive action. The reduction of thyrotoxicosis depends on the knocking out, or partial knocking out, of some of the parenchymal cells. The benefit in cancer is through the killing of cancer cells.

With any of the types of irradiation used the therapeutic effect is due chiefly to the beta rays. When x-rays impinge on tissue secondary electrons are liberated which act in a beta ray like way. I^{130} (the twelve hour isotope) and I^{131} (the eight day isotope) both emit beta and gamma rays. The gamma rays, however, are less important in the production of therapeutic effect.

For over a year now Skanse has been making studies on chicks and rats to discover the precise manner in which radiation of iodine affects the several functions of the thyroid. Briefly it may be said that when he uses in these small animals doses which in terms of the weights of their thyroids are comparable in amount of radio activity to that used for therapeutic effects in humans, he finds definite inhibition of the growth of the thyroid and impairment of its ability to collect iodine. The former effect precedes the latter. The capacity of the thyroid to respond to stimulation by thyrotropic hormone also is damaged by irradiation, but not as early as the two previously mentioned functions.

The advantages of radioactive iodine over the other forms of irradiation therapy which have been used are very great. When the thyroid collects iodine it is utilized by the thyroid cells with great rapidity, as shown by the work of Chaikoff and his coworkers,[13] to iodinate tyrosine to diiodotyrosine. The next step in hormone manufacture, which consists in the formation of thyroxine from diiodotyro-

sine, appears to take place in either cell or follicular lumen. The point of practical importance is that very soon after the administration of radioactive iodine, most of the radio activity is to be found in the lumen of the follicle, and in delineating the mechanism involved in this type of radio therapy, one should consider that the thyroid cells are being bombarded by beta rays originating from within the follicle. This situation is greatly in favor of radio iodine as a source of radiation. Beta rays have a low penetrating power, a few millimeters, and when they originate within the follicle, the parenchymal cells, which are the desired target, are the first to be hit, nor will the irradiation travel far from the target to injure structures that it is not desired to irradiate. Contrast these relationships with those of x-rays administered from without and having to traverse the surrounding structures of the neck before reaching, or after passing, the desired target. The difference is as between shooting with a modern precision rifle and a 17th century blunderbuss.

The first reports on results of treating Graves' disease with radio iodine were those of Hertz and Roberts[5] and of Hamilton and Lawrence,[14] both of which were reported at the annual meeting of the American Society for Clinical Investigation in 1942. Hertz and Roberts gave a preliminary report on ten cases of Graves' disease treated with radio iodine, and Hamilton and Soley reported on three.

In 1946 Hertz and Roberts[15] reported further on the first series of cases to be treated for Graves' disease with radio iodine. It consisted of 29 patients, all of whom were treated with the 12 hour isotope of iodine in doses varying from 5 to 25 millicuries of radio activity, given as a single dose, and followed then by a course of ordinary iodine. The reason for following the radio iodine with a course of ordinary iodine was twofold. On the practical side, it was to protect patients against mischief from thyrotoxicosis during a period in which a treatment of unknown efficacy was being tried out. I insisted on it for this reason. Also on the theoretical side it was postulated that perhaps the creating of a blood iodine barrier would retard the escape of radio iodine from the thyroid. Subsequently Chapman obtained evidence in seven cases that such an action does not appear to be exerted.

When Dr. Hertz left us for naval duty in 1943, Dr. E. M. Chapman took over the investigation of the possibilities of treating Graves' disease by means of radio iodine. First off he started a second series

of cases treated by I^{130}, the 12 hour isotope, and nothing else.[16] The average dose became 30 millicuries and the range was from 15 to 79. The policy was to give from 0.5 to 1 millicurie for every gram (guessed) of thyroid tissue.

This series now includes 65 patients. At the present writing (December, 1947) 46 of these have become euthyroid, 11 hypothyroid and taking thyroid, and 8 were improved but still thyrotoxic and 5 of these were subsequently treated with a second dose, of eight day isotope. Thus far no recurrences have been observed. No untoward effects have been noted other than transient röntgen sickness, and some swelling and tenderness of the thyroid. The goiters have all got smaller and about half of them have disappeared. As to the eyes, one can say that there has been no evidence of reduction in actual proptosis, but in most instances lid retraction has been reduced, and swelling of the lids has diminished. In no instance has the ophthalmopathy been aggravated following the exhibition of radioactive iodine.

Subsequently (1946), Chapman started a third series of cases of Graves' disease in which all patients were treated only with I^{131}, the eight day isotope. The dosage used was from 4 to 14 millicuries. This series now numbers 60 patients. Of these 36 have already made satisfactory responses. Three of them had second doses given. Twelve patients are better but still somewhat thyrotoxic six months after treatment, and eleven have been treated within six months, and it is considered too early to evaluate the results. One patient died of myocardial infarction 19 days after treatment, and in one other case no follow-up has yet been secured.

At this point some discussion of choice of isotopes is indicated. For therapeutic purposes we started with the twelve hour isotope because that was the only one available. We got it from Massachusetts Institute of Technology, and with it a very generous collaboration on all the physical parts of the program from Professor Robley D. Evans and his associates. When in 1946 it became possible to get radioactive isotopes from Oak Ridge, Tennessee, MIT discontinued supplying us with iodine, but continued to collaborate on preparation of doses and detection of radio activity in excreta. From Oak Ridge we only get I^{131}, or the eight day isotope.

The dosage, of course, of the two is different. I^{130} gives off its total radiation, so Dr. Skanse informs me, in three or four days, whereas I^{131}

takes from forty-eight to fifty-six days to do so. It is obvious then that a smaller dose must be used with the latter than with the former. But how much smaller? The work of Chapman and Evans[16] had shown that an effective, and not observably injurious, dose of I^{130} is 0.5 to 1.0 millicurie of radio activity for every estimated gram of thyroid tissue. This dosage had become rather well established and was giving good results when we were obliged to shift to I^{131}. Being fearful of causing some injury, the dosage employed with this isotope was in the beginning held at what was thought to be a conservative level, namely, 4-8 millicuries. It has been slowly increased to 8-14 millicuries.

The ultimate choice of isotope will depend on which offers the most benefit and subjects the patient to the least chance of harm. So far I have said nothing about harm, beyond noting that to date no serious injury has been identified. The theoretical types of harm of which we are most fearful, are the production of cancer of the thyroid some years after treatment, and injury to the kidneys caused by excretion of radioactive material. So far, no injury having been observed, it is impossible to make any choice of isotope from the point of view of likelihood of injury, nor is it yet possible to make a selection in terms of therapeutic effectiveness. It is true that to date our results with I^{131} are not as impressive as with I^{130}, but that may be merely because the method of using it is not so well developed.

In the field of cancer of the thyroid, experience is less than in Graves' disease. Our own opportunity has been greatly increased of late, however, because Dr. Rawson of our group, through the invitation of Dr. C. P. Rhoads, has been directing the studies on thyroid cancer patients also at the Memorial Hospital here in New York. In some cases neither the parent tumor nor metastases, either before or after thyroidectomy, take up any iodine, but out of 14 cases in which the distant metastases originally before thyroidectomy took up insignificant amounts of, or no iodine at all, in six after total thyroidectomy, either surgical resection or complete destruction of the goiter by radio iodine, the metastases increased in capacity to collect iodine sufficiently so that a therapeutic dose of radiation could be got into them via iodine.

In one out of three cases in which TSH was exhibited after total thyroidectomy for cancer, the metastases showed an increased uptake of radioactive iodine.

What the effectiveness of radio iodine in the treatment of cancer

of the thyroid may ultimately prove to be, cannot be foretold now. It is still in the experimental stage. Every effort should be made in all cases to make a total removal of the thyroid and its tumor by radical surgical resection. When this is impossible, all iodine collecting tissue can be destroyed by radio iodine in sufficient quantity. The great question, however, remains: can malignant thyroid tissue, by any maneuver, be induced to take up sufficient iodine to admit a total destructive dose? That at the moment is unanswerable. At least it can be said, however, we need have no hesitation in using radio iodine in the treatment of cancer as we do in Graves'. Cancer already being present, we need not worry about producing more later, nor is the evidence that renal damage may be produced sufficient to deter us. The only renal damage that Rawson has observed was in one cancer case, at the Memorial Hospital, in which, after the patient had received 180 millicuries of radiation, there was observed a 50 per cent decrease in glomerular function. After six months this returned to normal. Evidence that radio activity in thyroid or metastases injures other parts of the patient's body is lacking.

Protection of Workers

It would not be fitting to omit from this talk a word about the safeguarding of workers from the untoward effects of radiation. A technique must be worked out by all groups handling radioactive materials, and carried out with quite as meticulous care as is the aseptic technique of the operating room. There is this difference, however, the aseptic technique is for the patient's protection; the radiation protective technique is largely for the protection of the worker. Rules for health protection in handling radio isotopes have been issued by the Atomic Energy Commission* and should be followed insofar as the local situation demands, in any laboratory or clinic using such materials.

In our clinic at the present time here is what happens. Shipments of radio iodine are received by air mail from Oak Ridge, Tennessee. The material is in watery solution and each bottle of it is enclosed in an inch thick lead cylinder. These in turn are encased in strong wooden containers, all of which belong to the Government and have to be returned immediately. Such newly arrived material, or "hot stuff," is handled in appropriate glassware either at the end of strings or with

* U. S. Atomic Energy Commission, Isotope Branch Circulars B1 and B2, Oak Ridge, Tennessee.

tongs. All pipettings, etc., are done by distant control behind lead screens. An aliquot of all consignments from Oak Ridge is taken, duly screened to Massachusetts Institute of Technology for calibration. When this has been done, doses are made up by suitable dilution.

Doctors and technicians wear rubber gloves and lead impregnated aprons. They also wear badges containing x-ray film. Development of the film at stated intervals permits the exposure of the wearer to be measured; "0.1 rem. represents the total additive exposure from independent components of all radiation involved." Total exposure of an individual per day should not exceed this amount. Our people, we find, are not exposed to anywhere near this. Because of the concentration of iodine in the thyroid, the protection of radio iodine handlers is facilitated by examining their thyroids for radio activity with the Geiger-Müller counter, from time to time. We have not as yet found any activity over the thyroids of our workers.

The patient himself after the radio iodine is in him, is not a source of danger to others, at least if not closer to his neck or the location of metastatic cancer than ten feet. Also he would cease being dangerous even on direct contact after twenty-four days. His urine, however, is radioactive, and if it is collected, the specimens should be appropriately isolated.

There are fewer risks with radio iodine than with other longer lived isotopes. Nonetheless there are enough so that it behooves all concerned to take proper precautions.

Comment

It is not necessary for me to summarize or discuss further either the diagnostic use of radio iodine or the treatment of cancer by means of this agent. I am sure, however, that you expect from me at this time an opinion on the value of radio iodine in the treatment of thyrotoxicosis—how it compares with that of other types of therapy.

It is my belief that there is only one sound way to treat hyperfunctioning adenoma of the thyroid. That is surgical removal after proper preparation. Our problem, therefore, is restricted to the thyrotoxicosis of Graves' disease. In Graves' disease there is not always an indication to treat thyrotoxicosis at all. In certain phases of it there may be no thryotoxicosis, and under such circumstances the indications may be, particularly if the ophthalmopathy is severe or progressing,

to give thyroid.

When, however, thyrotoxicosis exists and presents an indication for treatment, then we need to know which offers the patient most—surgery, prolonged antithyroid drug therapy, or treatment by means of radioactive iodine.

I may say at once that for such a purpose, were there no fear of doing injury, treatment with radio iodine would be by all odds the best therapy. Its advantages are several. From the patient's point of view it is delightfully simple—one swig of a nearly tasteless watery solution. Hospitalization is usual for preliminary study of the case, but it is not necessary as far as therapy goes. There is no necessity for frequent and close surveillance for toxic side effects as is imperative in the prolonged use of antithyroid drugs. There is not the ordeal of an operation.

To what degree the doctor should worry about late untoward effects, no one at present can say. The proponents of radio iodine therapy brush them off quite light heartedly. The very fearful refuse to use the treatment at all. If one asks experts on the effect of irradiation on tissue as to the likelihood of late carcinogenesis, one gets different replies from different experts. One such expert, in whose wisdom I have much confidence, told me he wouldn't worry about any isotope with a half life of not over eight days. My own belief is that the prospect of radio iodine induced cancer in the treated cases in the years to come is slight, but no one can legitimately say that it is non-existent.

Under these circumstances I can perhaps best indicate to you my opinion as to the relative merits of forms of therapy for thyrotoxic Graves' disease by telling you how we are actually treating our patients at the present time.

First I may say we are treating none by prolonged use of any anti-thyroid drug. Most of our patients we still treat by thyroidectomy after preparation with an antithyroid and iodine. We are committed, however, to a long term evaluation of radio iodine therapy. For that purpose we are selecting patients in the age group of 45 years or over. The idea is that if carcinogenesis does result in certain cases, it will not be before twenty or more years. Hence the selection of older patients. That is our policy at present. We stand ready to change it at any time for what seems to us good and sufficient reason.

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CRITICAL EVALUATION OF THIOURACIL AND THE NEWER RELATED COMPOUNDS IN THE TREATMENT OF THYROID DISEASE*

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Six years have now elapsed since Mackenzie, Mackenzie and McCollum[1] published in *Science* their short note on the goitrogenic action of sulfonamides. In this and in their subsequent publications in 1942[2] and 1943[3] the Mackenzies were able to show that a number of substances including sulfaguanidine and thiourea were capable of producing hyperplasia of the thyroid gland with marked diminution in basal oxygen consumption; also that these changes were prevented or corrected by the action of thyroxin but were unaffected by increasing the content of iodine in the diet. They speculated as to the mechanism of action and came to the tentative conclusion that the thyroid enlargement was mediated by a pituitary which was receiving an insufficient supply of thyroxin from an hyperplastic but functionally inactive thyroid gland.

Their observations were confirmed and extended by Astwood and his co-workers[4,5] who were the first to apply this newer knowledge to the treatment of human thyrotoxicosis.[6] The development for which the Mackenzies and Astwood were chiefly responsible marked an epoch and introduced a new principle in practical therapeutics. For the first time it appeared possible to control disease by preventing with drugs the formation of a hormone.

Astwood's work attracted wide attention, and clinical data concerning the action of thiouracil, the first favored of the new drugs, accumulated rapidly and from many sources. It soon became apparent that this drug in doses which were not usually toxic could bring about a reversal of most of the undesirable symptoms of Graves' disease.[7] Tachycardia was controlled; systolic and pulse pressure attained normal levels;

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tremor and heat intolerance disappeared; the strength of muscles was restored and normal weight was recovered. The basal oxygen consumption was reduced; the level of cholesterol in the serum rose to normal or higher than normal levels. The frequently prominent creatin defect of hyperthyroidism was no longer apparent; nitrogen and calcium balance was restored. These remarkable benefits could be attained usually without perceptible increase in the size of the thyroid gland. Of all the manifestations of Graves' disease, exophthalmos alone was not adequately controlled. Convergence was improved. The stare with its accompanying lid lag and lid spasm was diminished or lost, often giving a false impression that the proptosis had receded. Actual exophthalmometric measurements, however, revealed that the position of the eyeballs was not improved and that indeed in many cases the protrusion was slightly but definitely increased.[8] In the presence of complications of hyperthyroidism, thiouracil was not contraindicated. The progress of thyrocardiacs and of thyrodiabetics who received the drug simulated that following thyroidectomy.

In those who could tolerate the drug, failures were few. Benefit might be prompt with return to normal conditions in a period of ten to fourteen days or might be delayed for three or even six months. Promptness of action seemed to be dependent upon the amount of preformed thyroxin which existed in the body at the time when thiouracil treatment was started. Large glands and those in which iodine had previously been given seemed to respond more slowly. Optimal effects were achieved early in many young individuals with small glands regardless of the original height of the metabolic rate or the apparent severity of the thyrotoxicosis. Benefit could be indefinitely continued by maintenance doses considerably smaller than those required to initiate control. Surprisingly, remissions were sustained in many patients long after the drug had been withdrawn.

Unfortunately it soon became apparent that thiouracil was a dangerously actionable drug.[9] Eruptions, febrile reactions, or granulocytopenia appeared in approximately 10 per cent of the cases and often were sufficiently disturbing to require discontinuance. Agranulocytosis with its always alarming accompaniments occurred in about 2 per cent of all patients treated. Its appearance seemed to be independent of the size of the dose or the duration of treatment but was perhaps more frequent when the drug was renewed after temporary withdrawal or during the

simultaneous administration of sulfadiazine. It often developed with frightening rapidity, sometimes within 48 hours after record of normal blood counts. It constituted a medical emergency during which, before the advent of penicillin and streptomycin, there was a mortality of more than 10 per cent. Later when the use of these antimicrobial agents helped to prevent invasion of the body by pathogens during a crucial period, it was found that not infrequently the state of agranulocytosis was self-limited and that in many cases spontaneous return of granulocytes to the peripheral blood could be expected in ten days to two weeks after withdrawal of the drug.

After several thousand patients with hyperthyroidism had been treated, some opinions could be expressed with considerable assurance. It was apparent that thiouracil was a very effective drug in the control of many cases of thyrotoxicosis, but that its use was limited because of toxic side reactions. The development of agranulocytosis in approximately 2 per cent of the cases was a terrifying possibility because it was unpredictable, might occur at any time during the use of the drug, and carried a considerable mortality even with the best and promptest treatment. Frequent blood counts did not constitute a sufficient safeguard. Indeed, protection against complete development of the complication rested solely on the directions of the physician and the obedience of the patient in reporting immediately any deviation of health whether it be a cold, skin infection, or indigestion. Such supervision and the need of constant contact with a physician limited the activity of patients. Management of a large number of patients who were receiving thiouracil was a source of perpetual anxiety to the physician. After three years of experience, it was the consensus of many of those most experienced in the use of the drug that whenever subtotal thyroidectomy was permitted and feasible, operation offered a solution which was quicker, more satisfactory both to patient and physician, and with optimal surgery equally safe.

Today thiouracil has no place in practical therapeutics unless it be in the extremely rare situation where more generally applicable antithyroid drugs prove unsuitable and where it might be desirable to utilize thiouracil as a substitute.

Propylthiouracil. The profession owes a great debt of gratitude to Astwood not only for his pioneer work in the clinical trial of antithyroid drugs but also for his meticulous pharmacological studies of some three

hundred compounds which from their chemical structure might be expected to possess antithyroid activity.[10] As one result of his investigations, he was able to suggest for clinical trial in 1945 6-N-propylthiouracil as a substance which when tested in rats appeared to possess ten times the potency of thiouracil and which when administered to twenty-nine of his thyrotoxic patients appeared to accomplish all that thiouracil could do but with no unfavorable side reactions.

If animal susceptibility had been taken as a criterion for human use, 40 mg. of propylthiouracil should have produced the same benefit as a presumably optimal 400 mg. daily dose of thiouracil. Astwood's previous experience with other derivatives of thiourea had taught him that trials in animals can not be applied too strictly to man. In the case of thiobarbital, man had proven to be three to four times as susceptible as the experimental animals. His initial observations with the clinical use of propylthiouracil led him to the belief that man was less susceptible than the experimental animals but that a dosage of 25 mg. twice or three times each day should be sufficient. Thus most of the early clinical trials by all observers were carried out at this level, which was eventually found to be inadequate in many cases. Perhaps the optimal dose has not yet been established and evidence indicates that there may be considerable variation in individual susceptibility. Astwood noted that some of his patients responded rather promptly to the relatively small doses he originally proposed. On the other hand, our present custom is to start treatment with daily doses of 200 to 250 mg. and to continue with this amount until normal conditions have been established. The requirement for maintenance must be determined for each individual. Ordinarily 50 to 100 mg. each day is sufficient, but some may require 250 mg. over long periods while others need only 25 mg.

Because of widely varying individual susceptibility, usage of propylthiouracil in these doses may occasionally result in myxedema. Astwood encountered hypothyroidism in eight of one hundred cases and on doses varying from 25 to 50 mg. per day at variable periods after the inception of therapy.[11] In the eighty-one cases treated in our clinic, there were four cases of myxedema, two of which were noted at the end of the period of intensive therapy. Both of the others developed while on a maintenance dose of 25 mg. which had been continued for several months following the initial intensive therapy.

Failure to control thyrotoxicosis with sufficient dosage of antithyroid

drugs, either thiouracil or propylthiouracil, is rare, and most of those reported have been in early series before sufficient experience in dosage and individual variation of response had been accumulatd. Lahey[12] has recently reported his experience in the use of antithyroid drugs as a means of preparation for operation. The series included three hundred and eighty-one treated with thiouracil, twenty-eight with thiobarbital, and two hundred and sixty with propylthiouracil. He states that he encountered no patient who could not be controlled if the drugs could be given in large enough dose over long enough time. Remarkable resistance may be encountered, however, as in one of our patients whose thyrotoxicosis has failed to yield with a daily dosage started at 250 mg. gradually increased over a period of 5 weeks and now maintained for 5 months at 900 mg. of propylthiouracil.

Toxicity of propylthiouracil. Information from the manufacturers of propylthiouracil indicates that the drug was distributed for experimental trial in 3000 to 4000 patients before its commercial introduction. The number since treated cannot be estimated but is undoubtedly large. Thus far no death attributable to the use of this drug has been reported in the literature or has come to the attention of the manufacturers.[13] In approximately 1000 cases in which details were made available to the manufacturers of the drug, no instance of agranulocytosis was recorded. Reports have appeared in the literature from Astwood concerning one hundred cases treated without serious intoxication,[11] from McCullagh[14] in whose one hundred and ten cases there was one instance of sore throat with a fall in the leukocyte count, and from McGavack[15] whose seventy-one cases included one instance of febrile reaction which led to withdrawal of the drug. In our clinic at the New York Hospital eighty-one cases have been treated without encountering intoxication sufficiently severe to require discontinuance. Since the drug has bcome generally available the manufacturers have obtained information concerning three unpublished cases in which the responsibility of propylthiouracil in the causation of agranulocytosis was authentically established. One other case was recently recorded.[16] In a recent report, Lahey and Bartels[12] gave their experience in the use of propylthiouracil in preparing two hundred and sixty patients for surgery. In one there was a febrile reaction, in four there was mild depression of the white blood cells, and in one severe agranulocytosis was mentioned but without details.

Other undesirable but minor symptoms have been encountered. These have included urticarial eruptions, pruritis, and headache. These have seldom been of severity sufficient to require withdrawal of the drug, or to interfere seriously with the management.

It would be surprising indeed if a drug as potent as propylthiouracil did not display some undesirable side effects or produce occasional serious intoxication, and its extraordinarily favorable record to date does not preclude the possibility of latent toxicity. Its relative safety when compared with thiouracil, however, has been clearly established.

Remissions. The continuance of remissions following the withdrawal of antithyroid drugs has been much discussed. Reports in the literature are confusing since they are based on variable data concerning initial and maintenance dosage, duration of treatment, as well as the age of patients, the type of goiter and the severity of the thyrotoxicosis. Percentages of relapse in different series vary from 20 to 80 per cent. Williams'[17] figures are impressive from the standpoint both of the number of cases and of the length of observation. Of one hundred and eleven patients, fifty-one appeared at the time of writing to be in no further need of treatment, forty-four had maintained remission for more than a year and thirty-three for more than 18 months following the discontinuance of the drug. It was of interest that 70 per cent of the relapses occurred within 2 months and 88 per cent within 5 months of the cessation of treatment and that only one patient relapsed after remission had been maintained for a year.

In our own series of patients treated with thiouracil we reported one of the highest figures, 76.6 per cent, for sustained remissions. Continued observation of the same patients over a period of 2 more years has now reduced the percentage to less than 50 per cent.

From the accumulated data some generalizations are permissible.

The administration of thiourea derivatives does not demonstrably modify the basic etiological factors which were initially responsible for thyrotoxicosis. When such factors remain potent and active, relapses are probable and should be expected more frequently and earlier than after subtotal thyroidectomy since the amount of gland tissue which may participate is greater. It now appears that a sustained remission rate of 40 to 50 per cent following the discontinuance of antithyroid therapy may be compared with one of 80 to 90 per cent following optimal surgery. There is evidence that sustained remissions are less frequent in

men. Williams' experience is striking and showed 18 per cent of twenty-one males and 52 per cent of ninety females. Relapse appears to be more frequent in severe thyrotoxicosis and with large goiters although many exceptions may be found in the literature. In Williams' series the age of the patient, the duration of the disease and the nodularity of the goiter appeared to have little relevance to the tendency to relapse.

No rules are yet established as to the optimal duration of treatment or the time of discontinuance of the drug. Perhaps those who can be maintained in normal condition on small doses such as 25 mg. of propylthiouracil can continue to do well without treatment. The ability to endure difficult life situations without development of thyrotoxic symptoms should be taken into account. Enlargement of the thyroid gland with or without bruit in the absence of hypothyroidism has been emphasized by Williams as an indication for the continuance of treatment.

With the advent of propylthiouracil and with continued experience indicating the harmlessness of continued treatment, the problem becomes less serious. Perhaps it will be found expedient to continue maintenance dosage in many cases indefinitely in a manner similar to the use of thyroid in myxedema. This practice introduced during the past few months in our own clinic has been found thus far both safe and satisfactory.

Combined use of iodine and the thiouracils. There is consensus that the administration of iodine previous to the exhibition of thiourea derivatives tends to delay the benefit of the latter. Although there are many apparent exceptions where prompt response was obtained, the trend was unmistakable. The suggestion was made early in the experience with antithyroid preparations that the simultaneous administration of the two drugs might also impair or delay the action of thiouracil. This has proven to be erroneous and it is now recognized that iodine and thiouracil can be given together both in preparation for thyroid operations and in continuous treatment. Pathological studies have shown that the use of iodine permits increased colloid storage in glands made hyperplastic by thiouracil and diminishes both the vascularity and friability of thyroid at the time of operation.[12] Note has been made that simultaneous use of the two drugs may increase the size of the gland, a tendency which is seldom apparent when thiouracil is administered alone. While undesirable, such enlarge-

ment is seldom obtrusive and does not ordinarily constitute a contraindication to the practice.

It now appears that thyrotoxicosis can be controlled by derivatives of thiourea in almost all cases which do not display toxic reactions and that in the use of propylthiouracil, immediate undesirable or dangerous side actions are so infrequent as to constitute little contraindiction to its continued use. Exception has been taken, however, in several groups of cases and possible dangers of long continued administration have been considered. It has been suggested that continuous use with the resultant hyperplasia may lead to the development of cancerous changes in the gland. Ward[18] and Hinton and Lord[19] on the basis of histological studies have proposed that every nodular goiter should be prophylactically removed with the corollary inference that antithyroid drugs should be used in nodular toxic goiter only in preparation for operation. Early studies in animals demonstrated the placental transmission of thiouracil with concentrations in placenta higher than in the blood of the mother and in the fetal blood approximately 50 per cent of the maternal. Hpyerplastic glands amounting to goiter with later developmental defects were found in the offspring after birth. Such observations justifiably led to a fear of administering the drug to thyrotoxic individuals during pregnancy. All of these exceptions deserve critical scrutiny.

Possible Carcinogenic Action

Bielschowsky[20] reported in 1944 that simultaneous administration of the carcinogenic agent 2-acetyl-amino-fluorene and ally-thiourea produced adenomas and malignant tumors in the thyroid glands of rats, an effect which could not be demonstrated by the use of either drug alone.

These observations inspired an editorial in the Journal of the American Medical Association[21] which raised the natural question as to the possibility of causing cancer by thiouracil treatment of older patients in whom some carcinogenic factor may be present. Relevant to this problem are recent experiments of Rogers, Asper and Williams[22] who administered 2-acetyl-amino-fluorene and thiouracil simultaneously to a series of 12 rats with resultant malignant tumors of the breast and other organs but with hyperplasia as the only detectable lesion of the thyroid gland. Possibly relevant also are the observations of Paschkis[23]

who studied mitoses by the colchicin technique during administration of thiouracil. He found that mitotic activity reached a maximum in 10 to 15 days, and became less marked with continuance until after 6½ to 7½ months of uninterrupted thiouracil treatment, few mitoses were demonstrable. More impressive is the record of patients treated over long periods with antithyroid drugs. The exact number who have received one or another of the derivatives of thiourea cannot be known but must now include many thousands, some of whom have taken the drugs over long periods. Those constituting the group reported by Moore from the associated hospitals have been under rigid scrutiny and have included a large number of nodular toxic goiters. It is significant that after more than six years of experience only one case of carcinoma has been mentioned. This instance which was mentioned by Payne[24] of Norfolk in discussion at the 58th Session of the Southern Surgical Association deserves attention. A woman of 34 with a typical syndrome of hyperplastic hyperthyroidism received 0.6 gm. thiouracil daily for six weeks and iodine for 10 days in preparation for thyroidectomy. The removed thyroid was said to show an area of carcinoma about 3 mm. in size located in the center of both removed lobes. The gland itself was hyperplastic without nodules. The criteria for the pathological diagnosis of cancer and the type of malignant change were not mentioned although it was stated that several outstanding pathologists had agreed that the stained sections showed carcinoma. The difficulties of positive diagnosis from such small areas in a gland where surrounding cells are displaying the mitotic activity of hyperplasia must be admitted. Evidence to date does not preclude the possibility that antithyroid drugs can induce neoplastic changes but indicates that they are extremely rare or clinically insignificant.

Use in Nodular Goiter

In much of the recent literature there is insistence that nodular toxic goiter should be treated surgically because of the danger of carcinomatous change in thyroid nodules. This opinion has been based upon histological examination of nodular thyroids removed at operation. Thus Brenizer[25] in North Carolina found 4 per cent of carcinoma in 2324 cases, Ward[18] in California 4.8 per cent in 3539 and Cole[26] in Illinois 7.2 per cent in 532. The viewpoint emphasizing the necessity of surgical removal of nodular goiter was dramatically presented by Hinton

and Lord[19] who compared the incidence of clinically unsuspected cancer in nodules of the thyroid with similar nodules of the breast on the necessary removal of which there is contemporary consensus. For the breast they found 6.7 per cent of seventy-five cases, and in the thyroid 7.6 per cent of one hundred and eighty-four cases. If this comparison can be taken at face value the situation is urgent and the indication for surgical removal is at least as great for nodules of the thyroid as it is for those of the breast. There is however an obvious objection to acceptance of such a thesis. Although the histological studies of surgically removed thyroid nodules indicate a frightening incidence, clinically recognizable carcinoma of the thyroid is rare. Older statistics concerning the incidence of thyroid cancer indicate a remarkable correlation with geographical location, 1.04 per cent for the goiter district of Switzerland (Kocher and deQuervain) and approximately 0.1 per cent in non-endemic areas in the United States. Wilson's[27] tabulation in 1921 of 74,335 cases autopsied in this country showed an incidence of 0.26 per cent of thyroid carcinoma. This is to be compared with the incidence of nodules in the thyroid gland which may be found in 80 per cent of autopsies in a goiter area and in 8 per cent in non-endemic areas.[28] Rogers[22] and his associates in their instructive review have calculated that by using the Cole's figure of 7.2 per cent of carcinoma in nodular goiter, one might expect more than 5 per cent of the population in a goiter region and more than 0.5 per cent in a non-endemic area to suffer from thyroid carcinoma. Their examination of diagnoses of 544,918 admissions to the Boston City Hospital, the Johns Hopkins Hospital and the Massachusetts General Hospital is illuminating. Only 74 diagnoses of malignant thyroid neoplasms were found constituting 0.0136 per cent of the total hospital admissions and 2.29 per cent of all the clinically recognized goiters.

Interesting also is the distribution of histologically recognizable malignant neoplasia in non-toxic and toxic nodular goiter. Cole's figures which indicate the most disturbing incidence, revealed 17.1 per cent in patients in the non-toxic group and only 1.2 per cent in the toxic nodular cases.

Comparison of pathological and clinical experience leads inevitably to the conclusion that a large number of the thyroid glands which appear histologically malignant do not become clinically recognizable. It also appears that the great majority even of the histologically recog-

nized cancers develop in non-toxic goiters and that the incidence even of histologically recognizable cancer is low in the toxic nodular goiter which is the point of discussion in relation to the use of antithyroid drugs.

Such evidence should not lead to neglect of the possibility of the development of cancer. It indicates however the relatively small risk and a careful weighing of this risk against that of operative interference. Furthermore it must be emphasized that in relation to cancer prophylaxis, the mere presence of nodules in the thyroid may be less important than the circumstances under which they occur. Nodules encountered in the glands of young people in whom the incidence is low, single nodules at any age, nodules in aberrant tissue and nodules in which recent growth has been observed should excite attention as possible malignancies and usually should receive prompt surgical treatment. Furthermore all patients with nodular goiter should be warned to report if differences in size of the gland or a portion of it are noted. Evidence at present however does not justify the dictum that all who have toxic nodular goiters should have prophylactic thyroidectomy. Indeed it appears that a large number of them can be treated safely and successfully with antithyroid drugs.

Use in pregnancy. Early fears concerning the dangers of using thiourea derivatives during pregnancy have not been justified by accumulating practical experience. Astwood reported four cases,[11] Williams[17] saw nine patients treated for a month or more of their pregnancy. In three the drug was given before fertilization and continued throughout the pregnancy. One patient was similarly followed in our clinic. In none of these cases was the infant found to have goiter or other defect that could be attributed to the treatment. In light of the early animal experiments, however, it would appear wise to limit the dosage during pregnancy to the lowest which is necessary for maintenance of the health of the mother. Williams has emphasized the desirability of weaning infants of mothers who are taking antithyroid drugs since they are secreted in considerable amounts in the milk.

It is as yet too early to judge of the definitive and optimal treatment of Graves' disease. For many years, subtotal thyroidectomy was the only measure which offered hope of control. In the hands of the most skilful surgeons the results achieved have been favorable indeed with an operative mortality of less than 1 per cent and the prospect of per-

manent remission following thyroidectomy in well over 80 per cent of cases. Now there are two other therapeutic expedients which afford adequate control. As yet the experience with each of them is less than with surgery. Difficulties of regulating dosage with radioactive iodine have not yet been overcome. Propylthiouracil has not been tried for a sufficiently long time to judge of end results not to permit complacence concerning the absence of toxic manifestations. If it is indeed as harmless as it now appears to be, it offers an extraordinarily satisfactory method of control. In comparison with surgery and radioactive iodine, it has one outstanding and precious advantage, that of reversibility. When one takes into account the highly variable functional activity even of normal thyroid tissue, the ability to permit or inhibit the production of thyroxin to meet changing needs and life situations would appear to be a matter of importance.

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CERTAIN CONSIDERATIONS IN THE APPLICATION OF ISOTOPES TO MEDICAL PROBLEMS*

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SINCE ancient times, astronomers have studied the fixed stars and have accumulated vast amounts of information about their behaviour and composition. Within relatively recent times it has been proven that several of the stars previously considered to be single heavenly bodies are in fact made up of two discrete bodies rotating about each other. The study of such astronomical twins has been a fruitful field of investigation and has yielded information about the behaviour of stars in general, information which might have been difficult, perhaps impossible to secure had these twins not been discovered. Without in any manner supplanting the other disciplines of astronomy, the study of these twins has added materially to the general body of astronomical knowledge.

If we now negotiate the well-travelled transition from the vastly large to the vastly small, we find an analogy to the discovery of stellar twins in the discovery of similar siblings among the atoms of the elements. Associated with the names of the Curies, Moseley and Aston came the realization that the naturally occurring atoms of many of the elements were not homogeneous but were in fact mixtures. Thus, whereas the atoms of a given element were all identical with each other in regard to the number and arrangement of their planetary electrons, and hence in their reactivities, as the chemist is accustomed to use this word, the nuclei of these atoms might differ from each other. This nuclear variation manifested itself in variation in the atomic masses of the several atoms, in view of the fact that the weight of an atom is concentrated in its nucleus, and it has now been established that virtually all of the common elements, each comprising one species of atom, chemically speaking, contain two or more sub-species of atom of different mass numbers. These sub-species of atoms, siblings, if you will,

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chemically essentially indistinguishable from each other but differing in mass, are the isotopes about which we shall hear this evening.

There are certain properties of the naturally occurring isotopes which should be stressed at this time. The nuclei of the isotopes of the elements of lower atomic weight which occur in nature are in general perfectly stable, they have no tendency to decay or undergo spontaneous transmutation, and they emit no energy, whether radiant or particulate. The relative abundances of the naturally occuring isotopes of any element are extraordinarily uniform, if one examines various samples of the same element obtained from different sources. Both of these characteristics find exceptions among the heavier elements of the periodic table. The several isotopes of any element enter the same chemical reactions and at very nearly the same rates, both in the test tube and in the animal.

These in general are the properties of which the investigator takes advantage when he employs isotopes in tracer studies. In order to undertake such a study, however, there must first be made available to the investigator a supply of the desired element in which the relative abundances of the several isotopes are far from their normal abundances. In other words, the isotopes of nature must be separated. Unfortunately, in most cases this is a very laborious, and consequently a very expensive procedure. In fact, except in the case of hydrogen, where it has been found practical to separate heavy water, deuterium oxide, from light water, hydrogen oxide, as a by-product of the ordinary commercial use of water in electrolytic reactions, the expense of separated samples of naturally occurring isotopes has been a deterrent to their wide use.

The list of naturally occurring isotopes has, over the past few years, been tremendously supplemented by the generation of man-made isotopes, isotopes which, in many cases, do not occur in significant concentrations in nature. These materials today are products of cyclotrons, as in Berkeley, or chain reactors, as at Oak Ridge, and differ in one very important regard from the naturally occurring isotopes which I have just described. In general, these products are radioactive, which means, structurally, that the nuclei of these atoms, in contrast to those of the majority of the naturally occurring isotopes, are unstable. As these radioactive atoms undergo decay and transmutation, their nuclei emit radiations of the same types as have been long recognized to arise from

radium and its degradation products. Each sub-species of atom, according to its own particular habit, emits either alpha or beta rays, often accompanied by gamma radiation. Alpha particles, you will recall, are particles of mass four, doubly positively charged; beta particles are in fact electrons, of a mass approximating zero and singly negatively charged; and gamma radiation resembles visible light, only of extremely short wave-length or high frequency. Whenever an atomic nucleus emits either an alpha or a beta particle, the residue which is left behind is necessarily an atom of a species of element different from that initially present; in other words, a spontaneous transmutation will have been effected.

It is characteristic of radioactive decay that the rate at which atoms of a sample of radioactive material undergo transmutation depends exclusively upon the number of radioactive atoms present at that time. This rate appears to be uninfluenced by such variables as temperature and pressure, and ordinarily chemical operations seem to have no effect upon the probability of a particular atom decaying at a particular time. From the mathematics of the situation it may readily be shown that the time required for the complete decay and disappearance of a sample of radioactive material approaches infinity, regardless of the rate constant of this decay. In describing radioactive materials, therefore, it has been found convenient to refer to the half-life, the length of time which will elapse before half of the material initially present will have undergone transmutation. This half-life is a constant quantity for any particular sub-species of radioactive isotope, though it varies widely from one isotopic variant to another, ranging all the way from a small fraction of a second to many thousands of years, in the extreme cases. In the limiting case, stable isotopes may be regarded as having infinite half lives.

In addition to finding a supply of concentrated isotope of the desired element, the investigator must also devise or purchase suitable equipment for the analysis of isotopic mixtures. In the case of the stable isotopes, ultimate reliance is placed upon the mass-spectrometer. In the exceptional case of hydrogen-deuterium mixtures, other methods, dependent upon some property related to the atomic mass, have been devised, but in all other cases, a mass-spectrometer is essential. This is a complex and expensive piece of electronic equipment, and, because of the cost of the commercially available units, is, in many laboratories,

of home construction. As its name implies, it distinguishes between the isotopic sub-species of an atomic species on the basis of the difference in mass.

For the analysis of isotopic mixtures containing a radioactive component, one takes advantage of the fact that the emanation from radioactive materials, whether alpha, beta or gamma, alters the electrical properties of the gas through which it is passing. Various electrical devices have been employed for the quantitative measurement of these changes, the most popular of which today is the Geiger-Mueller counter, backed by a suitable scaling circuit. Instruments of this type are widely available commercially and at relatively moderate price. Even though the precision of measure, with such instruments, is in general not so high as may be obtained with a mass-spectrometer, the availability of the instrument and ease in its maintenance and operation has resulted in many laboratories favoring the use of radioactive over stable isotopes.

One frequently overhears, among workers in the field, heated debates over the relative virtues of the stable and radioactive isotopes, and a few generalizations in regard to this argument may be appropriate at this time. Once it is decided what element is to be employed, the first question is: What isotopes of that element are available? Radioactive isotopes of more than half of all the elements in the periodic table may be obtained from Oak Ridge, whereas the list of stable isotopes that have been separated from nature in usable quantities is very brief. Of at least two elements of prime biological interest, however, no radioactive isotopes are at hand, and therefore, if one wishes to work with nitrogen or oxygen, one is forced to employ the stable isotopes which, fortunately, are available. When carbon or hydrogen is to be used, the worker has a choice of both radioactive and stable products, while with such elements as phosphorus or iodine, only the radioactive modifications can be obtained thus far.

Of the many problems to which isotopes have been applied, there are certain ones in which the radioactivity *per se* is an integral part of the procedure. In such problems obviously a radioactive isotope must be employed. The administration of radioactive materials in the hope of altering cellular processes incident to the radioactive emanation is a favored type of investigation. Among the fields in which this type of study has proven of great interest is in the control of neoplastic

processes and here the experimentor tries to take advantage of the fact that certain elements and certain compounds tend to concentrate in certain tissues as a result of the vital processes going on in these tissues. The hope in every case is that a suitable radioactive material will be found to concentrate inside the cell in which alteration is to be produced. If this is accomplished, two benefits are achieved. In the first place, injury to other tissues will be reduced to a minimum, and in the second place, in view of the extreme proximity of the source of radioactivity to the cell under study, the law of physics which relates the effective intensity of radiation to the reciprocal of the square of the distance from its source, will in good part have been circumvented. The studies which Dr. Werner will describe constitute a beautiful example of this approach.

Another type of experiment in which radioactive isotopes are essential is one which depends upon the technique of radioautography. This technique is particularly useful in studying the anatomical distribution of an element or a compound. It involves the administration of a material labeled with a radioactive isotope. Sections or blocks of tissues are then prepared and brought in close approximation to a photosensitive emulsion. With the complete exclusion of extraneous light, those points on the emulsion which are struck by emanation from decaying radioactive particles will become exposed, much as an x-ray film is exposed, and on subsequent development and fixation, will appear as black spots. Superimposition of the emulsion upon the histologically stained specimen permits the observer to ascertain at what points in the section the isotopic material which had been administered, has concentrated, because, at those points, the emulsion will be exposed. The technical details of this procedure leave a great deal to be desired, at the present time, but hopes for the future are high. It is of passing interest that the history of radioautography is really surprisingly ancient, that the earliest evidence of radioactivity was obtained by Becquerel when he noted that a sample of pitchblende produced a fogging of a piece of photographic film which was separated from it by a piece of black paper.

Yet another type of experiment in which radioisotopes are of peculiar usefulness depends upon the fact that, in select cases, it is possible to determine the distribution of a radioactive material in the body by superficial exploration of the surface of the body with a sensitive in-

strument like a Geiger-Mueller counter. Not only have the limits of the integrity of circulation in a gangrenous extremity been explored by this procedure, but the detection of metastatic tumors of the thyroid has also been accomplished in such cases where the metastasis retains the capacity to concentrate radioiodine.

In regard to tracer studies in the realm of intermediary metabolism, certain of the criteria for the selection of the most appropriate isotope for the job at hand have already been mentioned. One will have to consider the availability and cost of the needed isotope, and the nature of the accessible analytical instruments. The selection of the stable isotope of nitrogen by Dr. London, for the experiments which he will describe, was obviously dictated by the fact that no other isotope of nitrogen is known to occur.

An open choice is still available to the experimentor who requires isotopes of hydrogen or carbon. In each case both the stable and radioactive isotopes have found usefulness and the selection is often dictated by considerations other than those mentioned so far. Of the two radioactive isotopes of carbon, that of mass 11 is of limited usefulness in that its rate of decay is very rapid, its half-life is of the order of one half hour. It is suitable therefore only for experimental procedures which are completed in a relatively few hours. The isotope of mass 14 has a half-life of somewhat over 5000 years, and is therefore eminently suited for biological studies. Its great advantage over the stable isotope of mass 13 is the dilution which it will withstand before it is lost analytically. The stable isotope of mass 13 exists in nature as about 1 per cent of all samples of carbon, the remainder being the familiar carbon 12. If one starts with a sample of carbon enriched with carbon 13 to the greatest extent that has been achieved to date, and dilutes this with ordinary carbon a few thousand fold, the enrichment of isotope becomes indetectable even to the most sensitive instrument designed for its detection. The radioactive isotope of mass 14, however, may be diluted millions of times with ordinary carbon, and its concentration still measured analytically with relative ease. If therefore the protocol is one in which one may anticipate tremendous dilution of the material administered prior to its final isolation and analysis, one might be forced to select the radioactive in preference to the stable isotope.

There are assorted hazards incident to the handling of radioactive

materials, which, taken together, constitute the chief advantage of the stable over the radioactive isotopes. The most obvious situation arises from the refusal on the part of the Atomic Energy Commission to permit the use of carbon 14 in studies on the human subject at this time. This ruling is based upon the very long radioactive life of this isotope and the uncertainty of the biological duration of a carbon atom in the human body. In view of the cumulative nature of radiation injury, the authorities have come to the properly conservative conclusion with the result that, if one desires to employ isotopic carbon in human experimentation one is compelled to use the stable carbon 13. The stable material is of course free from any such restriction.

Hazards to the health of the experimentor, his colleagues, and incidental personnel such as scrub-women and plumbers, constitute a major problem. For protection from radiation injury one relies on two devices, shielding and distance. The nature and thickness of the shielding, the magnitude of the distance which should intervene between the operator and the material will be dictated by the nature and quantity of material handled and the maximal permissable daily dose of radiation which is considered compatable with safety. This quantity, which has been set at 0.1 roentgen equivalent man per day is presumed to be safe by roentgenological standards but there are some geneticists who are concerned lest even this small dose increase the mutation frequency of germinal tissue.

Out-and-out laboratory accidents are of course to be avoided and all precautions have to be taken to prevent contamination of laboratories as a result of spillage or breakage. More subtle is the hazard of volatile radioactive materials, such, let us say, as carbon dioxide containing carbon 14. Shielding and distance will no longer serve as a safeguard if such a gas escapes into the laboratory air. Monitoring devices are generally employed to inspect all likely sites of radioactive contamination from time to time, and exposed workers carry such monitoring devices about with them at all times.

Yet another hazard, perhaps not quite so grave, is the danger that, incident to radioactive contamination, the worker will arrive at an incorrect experimental conclusion. Since these radioactive materials will withstand almost astronomical dilution before all trace of them vanish, the cleansing of any surface which has been in contact with such materials becomes extremely difficult, and the danger is ever present that

radioactivity which has been ascribed to a sample of material was in fact due to an infinitesimal amount of contaminant adhering to the vessel from some previous use. It has even been stated that whole laboratories have been contaminated to such an extent as to render them completely unfit for further studies involving the measurement of radioactivity.

One of the most puzzling problems in regard to the use of radioactive isotopes is the question of the disposal of wastes. In the course of any such study, inevitably there are residues and tailings, and if these contain radioactive isotopes of relatively long life, what to do with them becomes a major problem. The usual methods for the disposal of wastes, incineration, burial, or discharge into the water-ways or the ocean, have sufficed for the present, but none of these would appear to be a proper long-term solution to the problem. It may be that no entirely satisfactory solution to this problem will be found until rockets capable of delivering these wastes to interstellar space become available.

When I was invited to participate in this program, I was asked, among other things, to "look into the future" in regard to the usefulness of isotopes in medical research. Of the usefulness of isotopes thus far, I need say nothing, as the two succeeding speakers this evening will, by their experimental and clinical results, amply justify the existence of this tool. With the greater accessibility of both isotopes and instruments, it is becoming evident that increasing numbers of laboratories and hospitals are adding this tool to their armamentarium and the time is not far distant when the handling of isotopes will be as widely disseminated as is the operation of a Warburg apparatus. It must not be imagined, however, that isotopes are a substitute for brains and that all problems immediately resolve themselves when the aid of isotopes is invoked. In certain types of problems isotopes will prove extremely valuable. In other problems isotopes will continue to be unnecessary and valueless. As in the past, the success of a biochemical investigation will depend primarily upon the insight of the investigator and upon his skill in the handling of biological materials and in the classical operations of synthesis, isolation, purification, and analysis.

AN EVALUATION OF VACCINATION AGAINST EPIDEMIC INFLUENZA IN MAN*

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SINCE the initial discovery of human influenza virus in 1933 by Smith, Andrewes, and Laidlaw[1] many fundamental investigations on epidemic influenza have served to provide a sound scientific basis for the recent development of an at least partially successful method for active immunization against the natural disease in man.[2]

While these basic background studies cannot be reviewed in detail in the time available this evening, it would, nevertheless, seem desirable to mention some of the high points by way of introduction to the main subject, namely, an evaluation of vaccination against epidemic influenza in man. These background studies may conveniently be divided into those which were carried out from 1933 to 1942 and those which have been conducted from 1942 to date, concurrently with the attempt of the Commission on Influenza of the Army Epidemiological Board[3] to determine whether a demonstrably successful and at the same time practical method of vaccination against influenza could be accomplished.

Following the initial demonstration by Smith, Andrewes, and Laidlaw[1] in 1933 that a filtrable virus in the throat washings of patients with influenza could be transmitted to ferrets and that the serum from recovered ferrets and convalescent humans would neutralize the infecting capacity of this virus, these important observations were promptly confirmed by Francis[4] in 1934 by recovery of strains of the virus from cases of influenza in Puerto Rico and Philadelphia. During this same year both Andrewes, Laidlaw and Smith[5] and Francis[4] demonstrated that the strains of virus recovered from patients by inoculation of ferrets could be adapted to Swiss mice by repeated passage, thereby greatly facilitating further studies of the virus and laying the groundwork for the subsequent development in 1935 of an accurate neutralization test.[6, 7, 8] By utilization of this test Francis[7] then showed that the

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British W.S. strain, the Puerto Rican PR8 strain and the Philadelphia strain were immunologically similar, thus establishing the etiological identity of epidemics of influenza occurring in widely separated areas at this time.

Equally important was the demonstration in this same year by Francis and Magill[9] that in patients with influenza acute phase serum failed to neutralize the virus while early and six months' convalescent serum protected mice against infection, thus showing that humoral antibodies developed in humans, as well as in ferrets, in response to infection with the virus. This observation, confirmed in 1936 by Smorodintseff, Drobyshevskaya and Shishkina,[10] and subsequently by many others, made possible numerous important immunological studies and a method for proving diagnosis, much more widely applicable than the recovery of virus by inoculation of ferrets.

Three further important observations were reported in 1935. The first was by Smith, Andrewes, and Laidlaw[11] that ferrets and mice could be infected only by way of the respiratory tract but not by subcutaneous or intraperitoneal inoculation. This fact was then utilized by Francis and Magill[9] to show that mice could be actively immunized against intranasal inoculation by means of subcutaneous or intraperitoneal injection of active mouse-lung or ferret-lung PR8 virus, thus establishing the principle that active resistance to experimental infection could be accomplished. The second observation was the successful cultivation of the virus in tissue culture medium consisting of minced chicken embryo in Tyrode's solution by Francis and Magill,[12] and the third, of great importance for subsequent work, was the demonstration by Burnet[13] that influenza virus could be adapted to grow on the chorioallantoic membrane of embryonated hens' eggs.

Further advances in 1936 were the development of a complement fixation test for determination of antibodies by Smith,[14] a more rapid method than the neutralization test in mice; the demonstration by Magill and Francis[15] that, though antigenically similar, nevertheless differences did exist among strains of virus from different sources; and observations of epidemiological import, by Francis and Magill,[16] on serum antibody levels in normal individuals of different ages, which showed that 49.2 per cent had sufficient neutralizing antibodies to provide complete protection in mice, 29.4 per cent partial protection and only 21.3 per cent no protection. The studies on variations in antigenic

structure, subsequently confirmed and elaborated by Burnet,[17] Magill and Francis,[18] Francis and Magill,[19] Smith and Andrewes,[20] Burnet and Lush,[21] Horsfall and Lennette,[22] Magill and Sugg,[23] and others, have become of obvious importance in the selection of strains for vaccines and in the interpretation of results obtained by vaccination.

In 1937 additional discoveries were made. The more important of these were, first, the report by Francis[24] of failure to recover virus or demonstrate a convalescent rise in antibodies to the PR8 strain in an epidemic in California in the early months of 1936, suggesting that this epidemic was due to a new and different virus, which four years later was proved to be the case; second, the studies of Francis and Magill[25, 26] which showed that on occasion, though not readily, the virus of influenza might be recovered by direct inoculation of human throat washings in mice, tissue cultures and on the chorioallantoic membrane of embryonated eggs; and third the experiments of Smorodintseff *et al*,[27] in which it was shown that normal human subjects with little circulating antibody were readily infected experimentally while subjects with relatively high antibody titers were resistant, thus demonstrating a relationship between humoral antibody levels and susceptibility or immunity. In harmony with these experimental results were the simultaneous observations of Hoyle and Fairbrother[28] comparing antibody titers in a population group before and after an epidemic, those of Francis, Magill, Rickard, and Beck[29] on 120 cases in the 1936-37 epidemic comparing acute phase titers with convalescent phase titers, which showed approximately a tenfold rise, and those of Rickard, Horsfall, Hirst and Lennette[30] in the 1940-41 epidemic, in all of which it was shown that the level of antibodies was an important factor in susceptibility or resistance. Included in the report of Francis and his collaborators [29] were observations bearing on the important question of duration of immunity. Two and a half to five months following recovery antibody titers had fallen on the average about 50 per cent below the early convalescent level. There was, however, considerable individual variation.

The preceding year, 1936, saw the initiation of studies on vaccination of man against influenza. Among these may be mentioned those of Chenoweth, Waltz, Stokes and Gladen[31] with human and swine viruses; of Francis and Magill[32] and Stokes and his collaborators[33, 34] with an active culture virus vaccine in 1937; of Stuart-Harris, Andrewes

and Smith[35, 36] and of Taylor and Dreguss[37] with a formalin-inactivated mouse-lung vaccine from 1938 to 1940; of Siegel and Muckenfuss[38] in 1940 with active virus; and those of Horsfall, Lennette, Rickard and Hirst,[39] of Dalldorf, Whitney and Ruskin,[40] and of Eaton and his collaborators[41] with a complex influenza-canine distemper vaccine[42] in 1941. Suffice it to say that while all of these vaccines, none of which was significantly concentrated, stimulated some rise in antibodies in the vaccinated individuals, there was no acceptable evidence forthcoming that they induced a sufficient degree of resistance to natural infection to warrant their use on a wide scale.

In the meantime, experiments by Francis,[43] reported in 1939, showed that, within certain limits, a direct proportional relationship existed between the concentration of virus used for immunization of mice and the degree of active immunity to intranasal infection induced, a result which of course suggested that a more concentrated vaccine than those being tried would be necessary to produce an effective immunity in man. As will appear later in the discussion, this suggestion has been found to be valid.

In November, 1940, Horsfall[44] published a review on the status of knowledge concerning influenza at that time. From this review I have culled certain items which it seems worthwhile at this point either to quote or to paraphrase since it may serve to emphasize the rapid progress which has been made since that time.

The first item relates to the etiology of influenza. Calling attention to the failure to recover a virus or demonstrate a rise in antibodies to the W.S. or PR8 strains of virus in certain epidemics in California in 1936, in England in 1939, and North Carolina in 1940, Horsfall endorsed the joint proposal from the International Health Division of the Rockefeller Foundation and the National Institute for Medical Research, Hampstead, London[45] that the specific disease caused by virus immunologically like the W.S. and PR8 strains be designated influenza A and that the terms influenza B, C, etc., be reserved for influenza caused by as yet unidentified and still hypothetical viruses immunologically distinct from A, if and when discovered.

The second item concerns the time required to identify an epidemic by recovery of the virus. I quote, "The recovery and identification of influenza A virus from a given throat washing require considerable time and even under the most favorable circumstances cannot be accom-

plished in less than three weeks."

Horsfall goes on to say that though Francis and Magill[25, 26] had succeeded in establishing the virus directly in mice, tissue culture and on the chorioallantoic membrane of the developing chick embryo without preliminary ferret passage, this had been successful in only a small proportion of cases, and that it should be emphasized that no rapid or simple method had yet been devised for the demonstration of influenza A virus in a given throat washing.

The third item concerns the time required to identify an epidemic by serological means. Pointing out that a significant antibody rise diagnostic of influenza A can be considered demonstrated only when the titer of the convalescent serum is four or more times higher than the titer of the acute phase serum and that antibodies reach maximum levels only within ten to fourteen days after the onset of influenza, Horsfall states, "Given ideal conditions, it is possible to obtain evidence of an increase in specific antibodies by means of the complement fixation test in from ten days to two weeks after the beginning of an epidemic. With the neutralization test a similar result can be achieved only within three weeks of the onset because of the additional time required for observation of the test mice."

And finally with respect to vaccination, with either active tissue-culture vaccine or inactivated mouse-lung vaccine, Horsfall concluded that there was "no convincing evidence . . . that either vaccine was effective in preventing the occurrence of the disease."

Hardly had this review by Horsfall appeared in print when Francis[46] reported the recovery and identification of a new type of virus obtained from patients in what appeared clinically to be an epidemic of influenza at Irvington House, Irvington on Hudson, in February and March, 1940. This virus, initially recovered by ferret inoculation, was adapted to mice by serial passage and by the 40th passage had become sufficiently virulent to kill regularly in a 1:1000 dilution. This strain of virus, called the Lee strain, was then shown by appropriate neutralization tests to be immunologically distinct from influenza A virus. It was further shown by the use of convalescent sera preserved from the previously unidentified February, 1936, epidemic in California that this epidemic was caused by a virus immunologically like the Lee strain. In accordance with the classification proposed by Horsfall *et al*,[45] the new strain was designated influenza B virus.

Almost simultaneously and quite independently Magill[47] reported the recovery of a new virus which was subsequently identified with the B virus by Magill.[48] Similarly by retrospective serological tests, Lennette, Rickard, Hirst and Horsfall[49] reported that epidemics in North Carolina and the West Indies in the Spring and Summer of 1940, respectively, were influenza B; Eaton and Beck[50] reported that cases in California in early 1940 were influenza B; and Nigg, Eklund, Wilson, and Crowley[51] that cases in Minnesota in May 1939 were due to the B virus. Thus influenza B was established as an etiologically distinct type of influenza, though clinically similar to influenza A.

The next new and highly important discovery was made by Hirst[52] in 1941 when he noted in the process of harvesting chicken embryo cultures that the red cells coming from ruptured blood vessels of chick embryos infected with influenza virus agglutinated macroscopically in the allantoic fluid within 15 to 30 seconds. Taking advantage of this observation, Hirst[53] then devised the hemagglutinin test for the quantitative determination of the amount of virus present in a fluid and the hemagglutinin-inhibition test for the quantitative determintaion of antibodies and the prompt differentiation of influenza A and B viruses. Hirst[53] further showed that the inhibition test for titrating antibodies correlated well with the neutralizing capacity of serums and also that the red cells under proper conditions not only adsorbed practically all the virus but that following adsorption elution took place, releasing nearly all the virus. These observations of Hirst may fairly be said to have revolutionized technics employed in the study of influenza and to have provided means for rapid identification of virus following the onset of an epidemic, for the extension by a readily performed and rapid method of studies on antibody content of serum, and one method for the concentration and standardization of vaccine.

Meanwhile, further advance had been made in the direct isolation of human influenza virus in chick embryos. In 1940 Burnet[54] reported direct isolation of seven strains by inoculation of throat washings into the amniotic sac. In 1942 Hirst[55] obtained twenty-eight positive results out of fifty-four filtrates of known positive throat washings which had been stored for fourteen to eighteen months at −76°C. In the November-December, 1943, epidemic of influenza A, Rickard, Thigpen, and Crowley[56] successfully recovered the virus by direct intra-allantoic inoculation of unfiltered washings in nine of twenty trials, four in the

first egg passage, and identified the epidemic as influenza A in forty-eight hours after the onset of the first case. Finally, in 1945, in a comparative study of various technics, Hirst[57] found that the inoculation of the amniotic sac with unfiltered washings to which 125 units of penicillin per cc. had been added was the most sensitive method, being successful in thirty-three out of forty-five trials.

The importance of the foregoing technics for the rapid recovery and identification of influenza virus, if vaccination is not undertaken until the appearance of a presumptive epidemic, is self evident, for as will appear later, immunity cannot be established in less than a week after vaccination.

With the foregoing background in mind let us now turn to a consideration of the development of the more effective vaccination against influenza which has taken place during the last five years, in an effort to evaluate the present situation and to point out some of the problems requiring further study for their solution.

Although Horsfall and his collaborators[39] had apparently obtained some success by vaccination with their complex chick embryo-canine distemper vaccine in 1940-41, with a decrease of approximately 50 per cent in the incidence of the disease among the vaccinated groups, the results were not consistent in all groups. In reviewing these results in a paper presented in April 1941, Horsfall[58] states, "Obviously these results do not indicate that a satisfactory and trustworthy immunizing agent against influenza A has been found. On the other hand, the reduction in incidence observed is not negligible and suggests that at least a step, however small, has been made in the direction of the eventual control of this disease. Furthermore, the results obtained tend to confirm the available evidence concerning the relationship between high antibody levels and relative immunity to influenza A and therefore may serve to point the way toward more effective prophylaxis. It seems possible that should a vaccine of considerably greater potency become available and should this improved vaccine increase specific antibodies to even higher levels, somewhat more striking reductions in the incidence of influenza A might be expected to result from its administration. Although these are intriguing problems for the future, it should be emphasized again that influenza A is merely one etiological variety of influenza. Until such time as those other unknown causes of the disease are discovered, it will hardly be possible to develop satisfactory

prophylactic measures against epidemics of influenza as such."

The next step, then, was an effort to develop a more potent vaccine by concentration of the virus and this was undertaken by a number of investigators. In 1942, Hirst, Rickard, Whitman, and Horsfall[59] in a comparative study of eleven different preparations of vaccine, ranging in strength from 36,200 to 214,000,000 50 per cent mouse mortality doses of influenza A virus per dose of vaccine and from 1000 to 1,000,000 50 per cent mouse mortality doses of influenza B virus per dose of vaccine, showed clearly that the strongest vaccines concentrated by high speed centrifugation were much superior to the weaker vaccines in stimulating antibody production and, in fact, induced a response quite comparable to that found in early convalescence from the natural disease. Subsequently Hirst, Rickard and Whitman[60] and Hare, McClelland and Morgan[61] reported independently that when infected allantoic fluid is frozen and then permitted to thaw, concentrated virus may be obtained by collection of the precipitate which forms. The mean antibody level two weeks after vaccination with this concentrate was as high as that which followed vaccination by similar amounts of virus concentrated by centrifugation.

Likewise in 1942 Francis and Salk,[62] taking advantage of Hirst's observation[53] on the adsorption on and elution of virus from chicken red cells, devised a simplified procedure for the concentration and purification of influenza virus and prepared an approximately ten times concentrated, mixed vaccine containing the PR8 and Weiss strains of A virus and the Lee strain of B virus. This vaccine also was shown to be antigenically more potent in actively immunizing mice and in stimulating higher antibody titers in humans than the unconcentrated vaccines previously tried. The main practical advantages of the eluate vaccine were considered to be that it could be maintained in fluid state at 4°C with unchanged potency for eighteen months and that it did not have to be rehydrated at time of injection. The major portion, though not all, of the inert chicken protein was eliminated.

With these more concentrated vaccines available and in the expectation that an influenza epidemic might occur during the winter of 1942-43, the Army Epidemiological Board's Commission on Influenza, under the direction of Dr. Francis, undertook the vaccination of groups of individuals with appropriate controls in various state institutions both with the frozen and thawed precipitate vaccine and the eluate

vaccine. Unfortunately, the prompt determination of the effectiveness of these vaccines in preventing the natural disease was frustrated by the failure of the expected epidemic to occur.

In the interval before the next epidemic, however, it was possible to conduct studies on the distribution of antibody titers at two weeks, four months and one year after vaccination.[63] These observations, which presumably have some bearing on the duration of immunity following vaccination, if expressed in the percentage of individuals having titers above certain levels, showed that for type A virus antibody titers of 256 or more were found in 15 per cent before vaccination, 83 per cent two weeks after vaccination, 69 per cent four months later and 66 per cent after one year, while for type B virus the corresponding figures were 6 per cent, 82 per cent, 70 per cent and 57 per cent, respectively. Francis,[64] Salk[65] and their collaborators were further able to show that individuals with antibody titers of 256 or more were relatively insusceptible to experimentally induced influenza A and B, when compared with those with titers of less than 256.

Even more important was their demonstration[66, 67] in a controlled study at the Ypsilanti State Hospital in Michigan that the vaccine had a significant protective effect against experimentally induced influenza A and B. In summarizing the results in the case of influenza A, the authors record[66] that clinical reaction with temperatures of 100°F. or more occurred in 50 per cent of the controls, 32 per cent of those vaccinated four and one-half months before, 14 per cent of those two weeks before, and 18 per cent of those vaccinated both four and one-half months and two weeks before challenge. None in the recently vaccinated groups had temperatures over 100.8°F. while 25 per cent of the controls and 11 per cent of those vaccinated four and one-half months before had fever of 101°F. or higher. Febrile reactions following exposure to virus occurred in 49 per cent of individuals having pre-infection antibody titers of 128 or less and in only 14 per cent of those having titers of 256 or more. Comparable results were obtained[67] with influenza B, although in this case the immunizing effect at four and one-half months was somewhat better maintained.

With this data at hand it was possible to set up in 1943 a well-controlled experiment in Army Student Training Program Units in nine universities throughout the country.[68,69] The participants in this investigation were Rickard, Thigpen and Crowley,[70] University of

Minnesota; Hale and McKee,[71] University of Iowa; Eaton and Meiklejohn,[72] University of California; Hirst, Plummer and Friedewald,[73] Princeton and Rutgers Universities and the College of the City of New York; Salk, Menke and Francis,[74] University of Michigan; and Magill, Plummer, Smillie and Sugg,[75] Cornell University and five medical and dental colleges of New York City. *In toto* between October 19 and December 4, 6,263 students were vaccinated subcutaneously with 1 cc. of a formalinized, allantoic fluid eluate, Type A and B, 10x concentrated vaccine, made up of the PR8 and Weiss A strains and the Lee B strain of virus; 6,211 comparable, alternate controls were injected at the same time with comparable material, excluding the virus.

Fortunately for the experiment, epidemics of influenza A developed in all these institutions in late November or early December. In the whole group, the incidence of influenza during the epidemic was 2.22 per cent in the vaccinated as against 7.11 per cent in the controls or a reduction in incidence of approximately 70 per cent. Expressed in another way 76.2 per cent of all cases occurred in the controls, 23.8 per cent in the vaccinated. Furthermore, the protective effect was comparable in all institutions except the University of California where the time elapsing between vaccination and the outbreak was considerably longer than in the others, approximately six to twelve weeks after vaccination from onset to termination of the epidemic. Whether this or some other factor was responsible for the less striking results at the University of California is uncertain. In the study at C. C. N. Y., because vaccination and onset of the epidemic were simultaneous, an opportunity occurred to show that resistance developed about eight days after vaccination.

It now remained to determine how effective this vaccine might be against an epidemic of influenza B and the opportunity occurred during the fall of 1945 at the University of Michigan, at Yale and in a small group at the Medical College of Alabama. Six hundred Army students had been vaccinated at Michigan on October 16, 1945, 550 at Yale, the majority on October 19 and a few early in November, and thirty at Alabama on December 4. For comparison there were 1100 unvaccinated Navy students at Michigan, 1050 at Yale, and ninety-five Navy and civilian students at Alabama. The results at Michigan, reported by Francis, Salk and Brace,[76] showed an incidence of influenza B among the vaccinated of 1.15 per cent, in the unvaccinated an incidence of

9.91 per cent. The results at Yale, reported by Hirst, Vilches, Rogers and Robbins,[77] were even more striking. There were 132 Navy cases or an attack rate of 12.5 per cent, only three Army cases or an attack rate of 0.5 per cent. At Alabama Friedman[78] reported two cases in the vaccinated group, 18 in the unvaccinated.

An analysis[79] of the data for the Army and Navy in the United States during this period would appear to be in harmony with the foregoing observations concerning the protective effect of vaccination against influenza B, the rate for common respiratory disease including influenza being in the Army, which had been vaccinated, approximately one half that in the Navy, which had not been vaccinated, a difference which did not occur during the influenza A epidemic in December 1943, when neither Army nor Navy personnel were vaccinated.

A similar satisfactory result has been reported by Norwood and Sachs[80] in a large industrial group. Among 360 vaccinated employees the incidence of influenza B was 1.94 per cent, among 4,280 unvaccinated employees 8.23 per cent, absenteeism being 4.4 x greater in the unvaccinated than in the vaccinated.

In summary, then, it would appear that in two adequately controlled experiments a considerable degree of success was attained in vaccination against the influenza A epidemic of November-December 1943 and the influenza B epidemic during the late Fall of 1945 under the following conditions: 1) when a potent formalinized and concentrated vaccine, in a single dose of 1.0 cc. subcutaneously and capable of stimulating an antibody response comparable to that which develops early in convalescence, was used; 2) when the antigenic structure of the strains of virus used in the vaccine was closely similar to that of the strains causing the epidemics; and 3) when vaccination had been carried out within 1 to 6 weeks prior to the onset of the epidemic.

Let us now review briefly the experience of 1946-47. On the basis of accumulated epidemiological experience,[81] which has suggested that influenza A epidemics occur every two to three years with sharper epidemics after the longer interval and that influenza B epidemics occur every four to six years, it was expected that an epidemic of influenza A might occur in the late Fall or early Winter of 1946-47. Consequently a number of large scale vaccination programs were instituted in several educational institutions and industrial companies.[82,83]

At the University of Michigan[84] 10,328 students were vaccinated between October 22 and November 2, 1946, with 7,615 unvaccinated students serving as controls. No epidemic appeared, however, until early March 1947, four months after vaccination. Between then and April 4 the incidence of acute respiratory disease was approximately the same in the vaccinated and unvaccinated groups, 7.19 per cent and 8.09 per cent, respectively. Subsequent studies by Francis, Salk and Quilligan[84] have shown that the virus concerned in the epidemic was apparently influenza A but that a sharp antigenic deviation from the standard PR8 strain was demonstrable. In the acute stage of the disease the mean antibody titer against PR8 virus was seventy-six in the unvaccinated, 499 in the vaccinated. In sharp contrast there was no difference in the low titers of vaccinated and unvaccinated against the 1947 epidemic strain. Ferrets inoculated with the new strain developed high antibody titers for the recently isolated 1947 strain but less response for the PR8 and Weiss strains of influenza A, while ferrets inoculated with the PR8 and Weiss strains exhibited high titers for these strains but little or no antibodies for the 1947 strains. The authors conclude that the failure of vaccination during the 1947 spring epidemic of influenza appeared to be due to lack of sufficient antigenic crossing between the strains in the vaccine and the prevalent epidemic strain.

At Yale[85] approximately 4,000 students were vaccinated during the fall of 1946. As at Michigan, there was no apparent difference in the incidence of influenza in the vaccinated and unvaccinated students when a mild epidemic of influenza appeared in late February and March, 1947, three to four months after vaccination.

Smadel[86] has reported a similar unsatisfactory result in the Army. Late in January 1947 outbreaks of influenza occurred at Fairfield-Suisun Army Air Base, California, Lowry Field, Colorado and Fort Monmouth, New Jersey. By serological tests these were identified as type A and on February 8 influenza vaccine was given to all military personnel. Subsequent epidemics occurred in a number of Army installations without any satisfactory evidence to show whether vaccination reduced the incidence or not. Strains recovered from patients at Lowry Field, Scott Field and Fort Monmouth were found to be antigenically closely related to each other but only distantly related to the standard PR8 and Weiss strains contained in the vaccine. Furthermore, the vaccine used did not elicit a good antibody response against the 1947 strain though

it did against the PR8 virus.

It has become quite obvious from the foregoing discussion that, despite the not inconsiderable success which has attended the work of the last five years on vaccination against influenza, many important, practical questions still require study and solution before a universally effective vaccine is at hand which will meet all the requirements of this difficult and complex problem of preventive medicine. To enter into a hypothetical discussion of these questions is probably not too profitable but nevertheless rather tempting. Suffice it, then, in bringing this discussion to a close, to ask some of these questions and answer them tentatively so far as possible on the basis of existing knowledge.

First, what is the best type of vaccine? At least four problems are involved in this question—antigenic effectiveness, toxicity, allergenic properties, and economic production on a mass scale. With respect to antigenic effectiveness, it would appear to be well established that formalin-inactivated and concentrated vaccine, containing approximately 0.2 to 0.25 mg. of virus material per cc., and irrespective of the method of concentration, is adequate to stimulate a satisfactory antibody response in approximately 85 per cent of individuals vaccinated, if a titer of 256 or greater be considered satisfactory. Various methods of concentration have been studied, including adsorption on calcium phosphate by Salk, [87,88] precipitation with protamine by Chambers and Henle,[89] freezing and thawing by Hirst, Rickard and Whitman[60] and Hare, McClelland and Morgan,[61] adsorption and elution from chicken red cells by Francis and Salk,[62] high speed differential centrifugation by Stanley[90] and by Taylor *et al*,[91] and methanol-precipitation followed by Sharples centrifugation by Cox, van der Sheer, Aiston and Bohnel.[92] Although the red cell eluate vaccine is the only one that has as yet been demonstrated by extensive, well controlled field trials to be prophylactically effective against naturally occurring epidemics, there is no reason to suppose, as pointed out by Salk,[93] that vaccines prepared by other methods of concentration would not be equally effective, provided they contain an equivalent amount of antigenically active virus. Though it is true, as emphasized by Stanley,[90] that a much more concentrated and equally antigenic vaccine can be prepared by high speed differential centrifugation than by the elution method, this is of little practical significance in so far as increasing the amount of virus per cc. of vaccine is concerned, since more virus per dose than

that in the eluate vaccine would make the vaccine too toxic for practical use, unless some method can be devised for detoxifying the virus without destroying its antigenicity. A somewhat different point of view, however, has been expressed by McLean, Beard and Beard,[94] based on their studies on the immunization of swine against swine influenza. Feeling that little further improvement is to be expected by increasing the dose, repeating the dose or by further concentration and purification of the virus from which the vaccine is made, they suggest, instead, that it would seem advantageous to direct more attention to the utilization of an active, attenuated virus, as described by Burnet and Foley.[95]

Concerning the problem of toxicity, which appears to depend upon the concentration of virus in the vaccine,[93] the reports in the literature appear to be quite conflicting with respect to the frequency of local and systemic reactions. Without attempting to analyze them in detail, it may be stated, I believe, that with the eluate vaccine the character and frequency of the reactions are fairly comparable to those seen with typhoid vaccine, annoying but not serious; that with protamine-precipitation vaccine the reactions are of similar character but reportedly less frequent;[82,83] and that neither kind causes absenteeism of consequence in industrial groups,[80,82,83] when compared with the absenteeism caued by epidemic influenza itself. It is to be hoped that future studies on methods of detoxification will lead to a reduction in the frequency and severity of toxic reactions, but in the meantime they would hardly seem sufficient to act as a deterrent to the use of the vaccines now available.

With respect to allergic reactions, which, as Ratner and Untracht[96] have shown, are related largely if not entirely to egg white protein in the vaccine, it would appear from wide experience that there need be little concern in adults, provided the essential precaution is taken to exclude from vaccination those known to be sensitive to eggs. In children, on the other hand, Ratner and Untracht[96] report on the basis of a careful study, that 1 in 200 may be sufficiently sensitive to require caution. They advocate an intradermal test with 0.02 cc. of vaccine, withholding vaccination in all cases of systemic reaction and giving the vaccine in three to six divided doses at one to three day intervals in cases showing only local reaction to the skin test. That it would be advantageous to reduce the amount of egg protein in the vaccine to a

minimal amount seems self-evident. From this point of view it would appear that vaccine prepared by differential high speed centrifugation would be preferable but even in the most highly purified preparations of PR8 and Lee viruses obtained from infectious allantoic fluid Knight[97] has shown that there is at least about 20 and 30 per cent, respectively, of an antigenic component characteristic of the sedimentable protein of normal allantoic fluid.

Concerning the problem of economical mass producton it is perhaps, as yet, impossible to make any categorical statement, but a combination of precipitation followed by high speed centrifugation[92] would appear to be the most promising.

Secondly, what is the best method of vaccination with respect to dose and method of injection. It would now appear established, through the controlled studies of the Commission on Influenza, subsequently confirmed by others, that a single dose of 1.0 cc. of the eluate vaccine given subcutaneously is adequate to produce a highly satisfactory antibody response and a high grade of resistance to infection.[63, 68] This dosage, however, is attended by more frequent toxic reactions than is desirable. Efforts to solve this problem are being made. Van Gelder, Greenspan and Dufresne,[98] in a comparative study in 1952 Navy personnel, have reported that a single dose of 0.1 cc. of vaccine given intracutaneously produced a higher rise in antibody titer than 1.0 cc. subcutaneously against both virus A and virus B, but this result as yet lacks confirmation and the degree of immunity against an actual epidemic provided by this method of immunization has not been determined. Until this is done, it remains an experimental procedure and cannot be advocated for general adoption.

More recently Salk[99] has reported that a single dose of vaccine given subcutaneously in amounts sufficient to be attended by a toxic reaction did not necessarily produce a higher antibody titer than somewhat smaller doses unattended by uncomfortable reactions. He has advocated for the present the largest possible dose of virus that can be given without systemic reaction. For adults this would be about one-fourth to one-half of the dose now being used. Here again these preliminary results must run the gamut of confirmation and a controlled field experiment before they can be adopted with confidence.

Thirdly, what is the actual effective duration of immunity following vaccination, when and how often should vaccination be done? Observa-

tions by Hirst, Rickard and Friedewald[100] on 2,945 individuals vaccinated 12-14 months prior to an outbreak of influenza A, when compared with a comparable control group of 4,451 persons, showed an attack rate of 3.6 in the vaccinated as against 5.5 in the controls, or a reduction of 35 per cent. Similarly Salk *et al*[103] have reported that in a group of 2,086 persons in Ypsilanti State Hospital, of whom 1,035 were vaccinated in December, 1942, and 1,051 served as alternate controls, the attack rates during an influenza A epidemic one year later were significantly lower in the vaccinated wards than in unvaccinated wards—in the female wards 3.4 per cent against 14.5 per cent, in the male wards 0.9 per cent against 9.7 per cent. It is further significant that, in the vaccinated wards of the hospital where only half of the patients had been vaccinated, this in itself seemed sufficient to check the spread of the disease to the unvaccinated controls in these wards. From the data available, then, it would appear that a considerable degree of immunity persists in a population group one year after vaccination. The tentative conclusion is that annual vaccination would seem the most desirable and practical procedure at present.

With respect to the most appropriate time of the year for vaccination, I believe it may be stated with considerable confidence that vaccination should be done prior to the appearance of an epidemic if the best results are to be obtained, for two reasons. The first is that it requires at least a week to establish immunity, the second that epidemics ordinarily spread so rapidly that it is impractical to undertake mass vaccination with the expectation that it can be carried through and immunity established before the epidemic has reached its peak in any given community. If this be accepted, October would appear to be the most appropriate time for vaccination since most epidemics occur in the late Fall or Winter months. The question naturally arises whether because of the periodicity of influenza vaccination should be practiced only in those years when an epidemic is expected. In this connection Francis[101] has said, "One of the chief obstacles to proper evaluation is the lack of cooperation on the part of the disease itself, in not presenting itself in the desired spot at the desired time." I would state it another way by saying that the chief obstacle is lack of knowledge which would enable us to predict with mathematical certainty when the next epidemic is going to occur.

Fourthly, what strains of virus should be used in the vaccine? Clearly

both A and B types of virus should be included, but what strains of each type? As pointed out above it has been well established by many investigators [102,103] that within each type a considerable degree of antigenic deviation may occur with the result that the strains included in the vaccine available at any given time may not be adequately protective for the strain to be encountered in the next epidemic. This difficulty was conspicuously illustrated by the unsatisfactory results obtained in the epidemic of last winter. Whether master strains can be found of sufficiently broad antigenic constitution to cover all strains within a type is a problem of continued research. In the meantime it would seem desirable to include with PR8 and Lee viruses, strains from the most recent epidemic and this is now being done by the inclusion of the FM1 strain of influenza A from the 1947 epidemic.[86]

Finally, concerning these and other problems awaiting solution, it is to be expected that final answers will be obtained only through continued research in the laboratory and the field. Certainly, it would not appear outside the realms of possibility that the resistance of the population as a whole may be raised and maintained at a level sufficient to forestall and prevent another disastrous pandemic.

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SECTION ON MICROBIOLOGY

FEBRUARY 18, 1948

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The Preparation and Properties of Purified Toxins and Toxoids

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Chemical investigations on bacterial toxins and toxoids are of practical and theoretical significance not only in the field of immunochemistry but also in the related preclinical and clinical sciences. The ultimate aim of such studies is to determine the nature, the character, the relationship between the biological and chemical properties, and the mode of action of toxins and toxoids.

The proper approach to these problems should concern first the determination of the physical, chemical and immunological properties of toxins and toxoids. In order to accomplish this, isolation of these substances in pure form is necessary. It is the purpose of this paper to discuss recent efforts to purify and characterize tetanal toxin and toxoid and diphtherial toxoid.

Both Eaton and Pappenheimer have prepared diphtherial toxin in a state of high purity. Groups of Army and Navy personnel at Camp Detrick have crystallized botulinal toxin (Type A). These investigators employed the classical methods of protein purification which involve the use of neutral salt precipitation, acid precipitation and adsorption. These procedures are largely based on the work of Denis and others which was carried out approximately a century ago, long before the chemical nature of proteins was understood. Although such empirical methods have yielded satisfactory preparations, it appears that these procedures leave much to be desired and that the problem of purifying toxins and toxoids should be solved by the employment of

precise methods which conform to the disciplines of physical chemistry.

Using the basic principles developed by Cohn and his associates for the fractionation of blood plasma in ethanol-water mixtures at low salt concentrations and low temperatures, the author and his colleagues have prepared crystalline tetanal toxin and highly purified staphylococcal and pertussal toxins. Pure diphtherial and tetanal toxoids have also been prepared. The methods employed involve the use of methanol-water mixtures under precisely controlled conditions of pH, ionic strength and temperature and have been reported in detail elsewhere.

Crystalline tetanal toxin has been characterized as a heat labile protein with an isoelectric point of 5.1 ± 0.1. The toxin has an electrophoretic mobility of 2.8 x 10^{-5} in veronal buffer of 0.1 ionic strength at pH 8.6 and has a sedimentation constant of 4.5 Svedberg units. Freshly prepared toxin has substantially constant solubility. The crystalline toxin gives the usual protein reactions, and contains 1 per cent sulphur and traces of phosphorus. It does not contain carbohydrate. The crystalline protein does not precipitate anti-*Clostridium tetani* rabbit serum. Four times recrystallized tetanal toxin contains between 3400 and 3600 flocculating units and about 6.6 x 10^{7} mouse minimal lethal doses per mg. of N. Crystalline tetanal toxin spontaneously converts to a flocculating atoxic dimer upon standing at 0° C. This change is accompanied by the appearance of another molecular species judged both by constant solubility test and ultracentrifugal analysis. The flocculating atoxic dimer has a sedimentation constant of 7 Svedberg units.

Pure diphtherial toxoid has been separated as a heat labile protein with an electrophoretic mobility of 8.1 x 10^{-5} in veronal buffer of 0.1 ionic strength at pH 8.6 and with a sedimentation constant of 4.6 Svedberg units. Its isoelectric point is 4.7 ± 0.1. The preparation has constant solubility and satisfies the existing criteria for a pure protein. It does not contain sulphur, phosphorus, carbohydrate or iron. The purified diphtherial toxoid does not precipitate anti-*Corynebacterium diphtheriae* rabbit serum. The final product contains between 2000 and 2200 flocculating units per mg. of N.

Purified diphtherial and tetanal toxoids obtained by the low temperature methanol method are now commercially available. They possess a number of practical advantages which may be summarized as follows. These toxoids which are more than 200 times purer than crude preparations generally do not elicit side-reactions when administered to children and adults. In general, it is no longer necessary to carry out reactor tests, and "booster" doses may be given without hesitation. The immunity obtained with these products is durable and of high order. The immunizing power of toxoids is potentiated by alum precipitation but unfortunately alum occasionally produces local irritation at the site of injection. Since the new preparations contain fewer solids, they can be precipitated with one-tenth the amount of alum required for crude toxoids. Side reactions to alum are therefore minimized. The addition of glycine permits sterilization by filtration and allows storage of these preparations for long periods of time under extreme temperature conditions without deterioration. Finally, since these toxoids are pure and stable they can serve as primary standards for the biological evaluation of products of equal or lesser purity.

The above mentioned advantages should lead to an increased use of toxoids for the prevention of diphtheria and tetanus. It should also lead to an intensified effort to purify by similar methods other toxins, toxoids and bacterial antigens.

*The Iron Enzymes of C. Diphtheriae and Their Possible Relation to Diphtheria Toxin**

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Studies on toxin production by C. diphtheriae have suggested that diphtheria toxin may be concerned in some way with the respiratory mechanism of the bacterial cell. Thus toxin is only produced under strictly aerobic conditions. In 1931, Coulter and Stone[1] showed that a free porphyrin is produced in culture filtrates in concentrations paralleling toxin production. This porphyrin has recently been identified as coproporphyrin III (Gray and Holt[2]). Finally both toxin and porphyrin are only produced in high yield when the iron concentration of the culture medium is very low.[3] In the case of the Toronto variant of the PW8 strain, for every 4 atoms of iron added over and above the optimum, 4 molecules of porphyrin and one of toxin fail to appear in the culture filtrate. Bacterial growth at optimal iron concentration for toxin production is about 80 per cent that obtained with excess iron. However, cells harvested from media containing an excess of iron contain 5-6 times as much iron as cells grown under conditions most favorable for toxin production. Cell suspensions of high iron content show a 2-banded hemochromogen type sprectrum when treated with sodium hydrosulfite and dilute alkali. These facts suggest that diphtheria toxin may be the protein moiety (or closely related precursor) of a respiratory enzyme. From the quantitative relationships between iron, porphyrin and toxin, the postulated enzyme should be the major respiratory pigment of diphtherial cells of high iron content.

The major hemin-containing pigment of *C. diphtheriae* is spectroscopically related to cytochrome *b* as was first noted by Fujita and Kodama.[4] It has been shown that cytochrome *b* is the limiting factor in the oxidation of succinate to fumarate by extracts of the diphtheria bacillus. The system which oxidizes succinate in these extracts is characterized by its insensitivity to cyanide, naphthoquinone SN5949 (Ball *et al*[5]) and carbon monoxide, compounds which inhibit the succinoxidase system in extracts from mammalian tissues. The differences between the diphtherial succinoxidase system and that of beef heart (Keilin and Hartree[6]), have been studied and can be explained by the presence in the bacteria of large amounts of cytochrome *b* relative to the other cytochrome components, cytochrome *c* and cytochrome oxidase, known to participate in the succinoxidase system.

These findings suggest the possibility that diphtheria toxin may interfere with the normal function of cytochrome *b* in the tissues of susceptible animals, possibly by blocking its synthesis.

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The Present Status of Immunization Against Diphtheria

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It is a pleasure to acknowledge the courtesy of the invitation to address the Academy and an honour to pay tribute to two of your illustrious members who have played such a prominent part in the control of diphtheria, namely William Halleck Park, and Béla Schick who is in the audience tonight. Time does not permit more than a very respectful nod to the past. It is to Ramon of the Pasteur Institute we owe the introduction of formol toxoid, a safe, easily standardized, inexpensive and very effective antigen for active immunization against diphtheria. The study of the effectiveness of toxoid in Toronto school children made at a time of high prevalence of diphtheria showed that three doses given at three weeks' interval resulted in a 90 per cent reduction as compared with the rate of their uninoculated school mates. That study initiated in 1927 had to be abandoned for the sole reason that the morbidity rate from diphtheria dropped to such a low figure and there maintained up to the present, as to make any comparison between the inoculated and non-inoculated meaningless.

Diphtheria has not been controlled by isolation and quarantine. Essentially, the control rests upon the simple principle of producing the most effective degree of active immunity in the greatest number of persons as early in life as possible and maintaining that immunity indefinitely. Administratively this looks like, and perhaps is, a formidable task. It has long been known that the response to toxoid and the loss of antitoxin are both subject to very wide individual variation. Both of these factors may be countered by giving the "dose de rappel" of Ramon, or more commonly designated recall or booster dose, in suitable quantity and at appropriate intervals. The basic principle of the secondary stimulus or recall dose has long been known. In 1898 Dean at Oxford showed the prompt and high rise in antitoxin in response to a secondary stimulus in a horse previously hyperimmunized and rested some years. Rufus Cole clearly enunciated that principle in 1904. He showed that a secondary stimulus in itself too small to elicit a detectable antibody response caused a rapid rise in agglutinins in an animal previously immunized and whose antibody titre had fallen to zero. More important perhaps than any other single factor in the control of diphtheria is the intelligent use of the recall dose.

There is a close analogy between active immunization against the diseases tetanus and diphtheria. Their respective toxoids readily call forth an antitoxic immunity which may be maintained at a high level when recall doses are given. This is perhaps best illustrated by the experience in the armed forces in regard to tetanus which was virtually eliminated in the recent war, one of the brilliant achievements of preventive medicine. The essence of this success lay in the fact that a recall dose of toxoid was accepted as a routine procedure. The unpublished results of tetanus antitoxin titrations of blood samples of some 2000 members of the armed forces indicate that all who had received the routine inoculations of toxoid, including the annual recall dose, showed a protective level of antitoxin ($>1/100$ u/cc). In contrast, in a small group of 53 who had not had a recall dose, only 62 per cent showed antitoxin at or beyond this level.

The effectivenes of minute doses of diphtheria antigen when given as a secondary stimulus is illustrated by the fact that a Schick test with control of diluted toxoid will produce a conversion from Schick positive to Schick negative in approximately 70 per cent of persons. The combined value

of the test toxin plus control is only 0.021 Lf. A secondary stimulus of Schick test toxin alone which represents only 0.001 Lf may give on the average a tenfold increase in antitoxin. To a group of twelve persons, all of whom possessed antitoxin initially, a secondary stimulus of 4 Lf of toxoid was given and blood samples taken daily for one week and at longer intervals thereafter up to six years. The first detectable increase in antitoxin was manifested by the fourth day; by the seventh day all had responded and the maximum titre was attained by the majority by the eleventh day. The highest level recorded in this small group was 120 units per cc. of serum. In a group of 340 persons, 95 per cent showed a response to toxoid when given as a secondary stimulus. These and similar studies were undertaken to determine the minimum, and at the same time practical, recall dose and to follow the level of antitoxin over a period of years. In general, the response in antitoxin varied with, though not in direct proportion to, the strength of the stimulus. In pre-school children where sensitivity to toxoid is not a problem, a recall dose of 20 to 40 Lf is desirable. In school populations, in order to avoid the necessity of a preliminary sensitivity test, 3 or 4 Lf of toxoid, because of its freedom from untoward effects and the satisfactory degree of antitoxin response, may be recommended as a recall dose. A similar dose is effective in adults, but except in the face of an epidemic, screening by a reaction test for sensitivity is desirable.

The rate of loss of antitovin as already mentioned is subject to wide individual variation. On the average, in a non-diphtheria environment, there is a loss in antitoxin of 60 per cent within two years as shown in a group of children studied in 1937. That is to say, taking the average antitoxin level of a group of immunized children as 0.33 u/cc, the average unitage has dropped to 0.13 u/cc in two years. Other studies have shown that from 10 to 30 per cent of persons revert to Schick positive within three to five years. It is quite apparent then that no success in the control of diphtheria may be expected without the recall dose.

The quality of toxoid is adequately safeguarded by the National Institute of Health. One may thus generalize by saying that any diphtheria toxoid given in two or three doses with an interval of three to six weeks may be expected to act as an effective primary stimulus. A recall dose given six to twelve months later will result in a protective level of antitoxin in well over 90 per cent. Without at least one recall dose the immunization procedure must be regarded as incomplete. In children immunized in infancy a second recall dose is recommended between the ages of eighteen and twenty-four months and a third when the child enters school. Possibly the second may be omitted, but because of the increased hazard of school a dose should be given at this time. In older children a booster dose is recommended every four or five years. In the armed forces approximately 50 per cent were Schick positive. In the recent epidemic at Halifax 45 per cent of cases were over fifteen years of age. Before diphtheria is effectively controlled adults will be required to be immunized. The administrative difficulties as well as the problems of reactions to toxoid are obvious. A preliminary screening with a Schick test and control of diluted toxoid (0.2 Lf/cc) which serves also as a "reaction test" is essential. Following this scheme, some hundreds of thousand personnel of the Royal Canadian Air Force were inoculated against diphtheria.

The use of multiple antigens will in some measure reduce the administrative burden of immunization. In a small series of very young children the response to diphtheria and tetanus toxoids was particularly striking after four doses of these antigens with pertussis vaccine added. There is good laboratory evidence to show that the bacterial element acts as an adjuvant.

With 600,000 cases of diphtheria per year reported in Europe there is no basis for complacency. Nothing less than a vigorous campaign of active immunization with a schedule of inoculations possibly more rigorous than necessary, is required to offset the menace of diphtheria in America.

*Immunization of Adults with Diphtheria Toxoid**

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Although immunization of children in the first years of life is rarely attended by serious reactions, the proportion of undesirable reactions following administration of diphtheria toxoid rises markedly with increasing age. These reactions in the older age groups may consist of severe local swelling with edema and tenderness accompanied by fever and malaise and are analogous to those caused by the injection of relatively large amounts of tuberculin into tuberculous individuals.

The more severe reactions to the subcutaneous injection of large amounts of toxoid, as well as the pseudoreactions observed in the Schick test have been variously ascribed to (a) constituents of the culture media, (b) to diphtherial bacillary proteins other than toxin or toxoid, (c) to toxin or toxoid itself. It is of practical importance to determine which of these constituents of the formalinized culture filtrates cause undesirable reactions in sensitive individuals. This is of particular importance at the present time because of the marked increase of the incidence of diphtheria among the older age groups.

We have, therefore, studied the reactions of adults to certain formalinized protein fractions from culture filtrates of the diphtheria bacillus. The materials used have consisted of:

1) A formalinized mixture of atoxic diphtherial proteins, hereafter called the P-fraction, which contains less than 0.03 per cent toxoid.

2) Highly purified diphtheria toxoid estimated to contain less than 5 per cent P-fraction or other nitrogenous material, prepared from highly purified diphtheria toxin previously shown to be homogeneous by physicochemical and immunochemical methods.

3) Crude toxoid prepared from toxic filtrates of organisms grown on a chemically defined medium and containing about 65 per cent P-fraction and 35 per cent toxoid.

The possibility of reactions caused by broth constituents has been eliminated by cultivation of the organisms on the peptone-free, chemically defined medium of Mueller and Miller.[1]

By means of two sensitive biological tests, the precipitin reaction and active and passive anaphylactic response in guinea-pigs, it has been demonstrated that purified diphtheria toxoid and P-fraction are immunologically distinct, one from another.

One hundred and eighty-six medical students and nurses were Schick tested with Schick toxin prepared by the New York City Bureau of Laboratories. Purified toxoid and P-fraction diluted to Schick strength in borate buffer containing 0.02 per cent gelatin were used as controls.

The reactions observed were found to fall into eight distinct categories exclusive of occasional reactions attributable to the stabilizing agents present in the diluents. Individuals may be Schick positive and show allergic reactions of the delayed tuberculin type to either P-fraction or toxoid, to both, or to neither. Similarly, individuals in the Schick negative group may be aller-

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gic to either fraction, to neither, or to both. Thus the materials show the same specificity in the human as shown by precipitin tests and anaphylaxis in the guinea-pig.

Among the Schick positive group only 3 of 58 were highly sensitive to purified toxoid. The sensitive individuals show some circulating antitoxin (ca 1/200 unit/cc.). Almost all of the Schick positive group, however, showed sensitivity to P-fraction and crude toxoid provided they were injected in high enough dosage. It is obvious, therefore, that the use of purified toxoid (purified especially with respect to P-fraction) rather than crude toxoid for immunization of most *but not all* Schick positive individuals should possess distinct advantages.

Since approximately 30 per cent of the Schick negative individuals showed allergic reactions to the toxoid itself diluted to Schick strength, we must conclude that even if toxoid were *completely* freed from last traces of impurity, its administration undiluted to an adult population without preliminary screening tests would result in a very high proportion of undesirable general reactions.

The Schick positive group were immunized with three doses of the purified fluid toxoid (0.002 cc. or 0.2 cc., 1 cc. and 1 cc.) administered at 3-week intervals. Because of preliminary screening out of sensitive individuals by means of reactions to the Schick test, no significant local or general reactions were encountered.

The antitoxin levels were followed during the course of immunization. Analysis of the results has indicated that delayed allergic reactions of the tuberculin type to either P-fraction or purified toxoid are indicative of "latent immunity." Allergic individuals of this type, particularly those sensitive to toxoid itself show a large anamestic response following injection of even very small doses of purified toxoid (0.002 cc or less).

This work has led to the conclusion that purified diphtheria toxoid provides a distinct improvement over the crude toxoid in current use. However, its advantage is limited essentially to Schick positive individuals showing no sensitivity. Since a high proportion of Schick positive adults show evidence of "latent immunity" to diphtheria and produce a high titre of antitoxin in response to very small doses of toxoid (see also Edsall[2]), is is questionable whether immunization of older children and adults with the full dosage is necessary or advisable in most cases. It is of interest in this connection that of 81 Schick positive medical students and nurses Schick tested with purified diphtheria toxin and with 0.0002 cc. (0.008 Lf) of purified toxoid as control, only 37 (45.7 per cent) were still Schick positive when retested one month later. The full immunizing dose of undiluted purified toxoid can be injected into these Schick positive individuals after the second Schick test without fear of undesirable reactions.

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